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### EXAMPLE 32

#### CONSTRUCTION OF 2H7 scFv IgG FUSION PROTEINS WITH HINGE MUTATIONS

5 A 2H7 scFv IgG fusion proteins are constructed with the first cysteine residue and the second cystein in the IgG1 hinge region substituted with a serine residue to provide MTH (SCC) and MTH (CSC). The template for introduction of the mutations is a polynucleotide encoding 2H7 scFv WTH WTCH2CH3 (SEQ ID NO:\_\_\_). The oligonucleotide introducing the mutations are 5' PCR primer oligonucleotides HlgGMHcys1 (SEQ ID NO:\_\_\_) and HlgGMHcys2 (SEQ ID NO:\_\_\_). The constructs are prepared as described in SEQ ID NO:\_\_\_). The encoding polynucleotides of the mutants are presented in SEQ ID NOs:\_\_\_ and the polypeptide sequences are provided in SEQ ID NO:\_\_\_).

Additional representative sequences of the present invention are as follows:

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## HuIgG1 wild type hinge, CH2, CH3

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tcgtgacagagaccacaatctgttgacanaactacacatgccaccgcgtgccagcaccgtgaactctggggggaccgtcagcttctctct  
cccccaaaaccagaagcacaccfcattgatctccggagcccttgagcttcacatctggtgtgtggcagcgtgacacagaagaccctgag  
gtcaagttaactgtgacgtgagcggcgtggaggtgcataatgccagaacaagccgcggcagggcagctacacagcagctacccgtg  
tggcagcagtcctaccgtctgtcaccagagctgctgtaatggcaagaggtacaaagtgcgaagcttcacaaaagccctccagccccc  
atcgagaaacaatctccaaaggcagcccgaggaacacagcgtgttacacctcccccactccgggaatgagctgaccaga  
accaggtgacgcctgacctgctgtcctcaaaaggctctatccagcagatctccgtggaggtggaggaacaatggcagccgggaacaa  
ctaacagaccacgctccctgtgtgcagctccagcggctctcttctctacagaacgtcacctgtgacaagaagcgtggcagcaggg  
gaacgtctctctgctgctgatgatgagctctgcacaaacctacacagagaagcctctccctgtctccgggtaaatgatctaga

*HuIgG1 wild type hinge, CH2, CH3*

sdqepksodkthtpccpapelggpsvlfppkpkdltmistrtevtcvvdvshdepevkfhwydgvge  
hnaaktprreeqynstyrsvsvtlvhqdwlngkeykckvsnkalpapietiskakgqprepqvydppsrdeltnqkvslctelvkgy  
30 psdiaewesngqpennyyktpvpsldsgsflyskitvdksrqwqngvfscsvmhleahnhytqklsislspgk

*Llama IgG1 hinge, CH2, CH3*

tgatcaagaaccacatggaggatgcacgtgccncagtgcccncaatgccngcncngaacnccaggaggc  
cctctgtcttctctcccccgaacccaaggacgtcctctccattttggaggccgagtcacgtgcgtttagtggaacgtcggaagaaa

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gaccocagaggtcaatttcaactgtgtatgtgatggcgttgagggtgogaacggcccaatcagaagccaaagaaggaaacaggticaacagcacg  
taccgcgtgtgtagcagctctgccattccagcaccaggaactggctgacggggaggaatcaagtgcaaggtcaacaacaagaactctcc  
ggccccatctcagagggaccattctcaaggccaaaggcagaccgggagccgcaggtgtacaccttggccccacacggggaagac  
tggccaaaggacacctgtgagctaaactgctgggtcaaaaggctcttaccacgtgacataacagttgagtgccagaggaaacggtcagccg  
5 ggticagaggcgacctacgccaaacagccggccagactggacaacgacgggacctacttctctacagcnaagctctctgttggaaga  
cagcgtgtagtgcagcaacttaactctgtgtgtgatcatgagggccctgcacaaccactacaaccgaatcaatcaaccacgctt  
ccgttaaatgataatctaga

**Llama IgG1 hinge, CH2, CH3 (In figure 23 as Llama IgG1)**

10                   ephggctcpqcpapelpgggsfvfppkpkdvlslsgrpvtcvvvdvgkedpevnfnwyidgvevrtantk  
pkeeqfnstyyrvsvlpqhgdwltgkefkckvnnkalpapiertiskagkgtrepqvytlaphreelakdvtvctlvkgfypadin  
ewarnqesegtvantpaldndgtvfyvsrlsvgektwqrgetltzvmhhealhnhtqksitassgk

## Llama IgG2:

15 tgatcaagaaccacagacaccnaaacnacnaccacacccacaaccaatctcacaaagaatccaagtgtcccnaaftgccag  
ccccctgagctctgggaaggcccctcagcttcatctcccccgaanaaccaggacgtctctcattcttgaggagcccgagggtcacgtg  
cgtgttgttagacgtgggcgaagaagaccggaggtcagtttcactgtgatagtgctgagctgagcgaacgccacacagagggc  
caanaaggaggaacgtttacacgcacgtaccgctggtgtcagctgcttccatccgcacagagactggtgacggsgaaggaattcaa  
gtgcaaggtaacaacaagaagctctccggccccatcagaagaacctctcaaggccaaaggcgagAACGCCGAGGTGTA

20 caccctggccccacaccgggaagagctggcAAGGACACCgtgagctgaatactgctgtcaaaaggctcttaccactgatatcaacg  
ttgagtgccagaggaattgggcagccgggtctagagcgacacacacccacccacccagctggacaacgacgggaacttctct  
ctacacgaagctctcgtgtggaacgaacagctgtgagcagcagcaggaacaccttaccctgtgtgtgtagcagaggcctgcacacact  
caaccacgaatctcgaagactcttcgggtggtgtaaatgtaattcaga

## 25 Llama IgG2

Dqepktpkppqpqpnpptesckpcpapelggpsvifppkpdvlsisgrpevtcvvvdvgqedpev  
sfnwiyidgaevrtantprkeeqnfstyrvsvlpiqhqdwtlgtckfkccvnnkalpapiektiskakgqtrepqvytiaphreelakt  
vsvtlvkgfypndinvearnqapesegvatppqldndgtfyflsksvgkntwqqgetfcvnmhealnhhytksitassgk

## 30 Llama IgG3 Fc

tgatcaagcgcaccacagcgaagacccagctccaaagtgtcccaatgccaggccctgaactcttggaggggcc  
cacggtcttcattccccgaaagccaaggacgtctctccatcacccgaaaacctgaggtcacgtgcttgtgtgacgtgggtataag

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gcacgtaccggtggtcagcgtctctgccatccagcaccaggactggctgacgggaaaggaaatcaagtccaagtcaacaacaaagt  
ctccagccccatcgaggaccatctccaaggccanaaggcagaccggagccgaggtgtacacctggccccacaccgggaa  
gagctggccaaaggacacctgtagcgtaacctgctggtcaaggcttctccagctgacntcaacgttgagtgccagaggaaatgggca  
gccggagtcagaggccacctacgccaacacgcccacagctggacaacgacgggacctacttctctacagcaaatctccgtggga  
5 aagaacacgtggcagcaggggagaagtcttcacctgtgtgtgatgcagggctctacaaatcactccaccagaaatccatccaccg  
tcttcgggtaaatgtaatactagaggccc

# Llama IgG3 Fc

dqahhsdpsskcpkcpellggptvfifppkakdvisitrkpcvtclwwtvvkdtrssswsvddtevhia  
10 etlqpkeeqfnstyrsvslpiqhdlwtlgkefkckvnnkalpapiertiskakgqtreppqvytlaphreelakdvtvstclvkgffpadi  
nvewqrngqpesegtyantppqldndgtyflsksvgkntwqqgevtcvvmhealhhnstqksitqssgk

## 15 *HuIgG1 wild type hinge*

gatcaggagcccaaatctgtgacaaaactcacacatgccaccgtgcccgca

## *HuIgG1 wild type hinge*

dqepksedkhtcpcpa

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HuIgG1 H2, wild type hinge with leu at second position (results from BglI site)

gntctggagcccaaatctgtgacaaaactcacacatgccaccgtgcccgca

HuIgG1 H2, wild type hinge with leu at second position.

25 dlepkdsedkhtcpcpa

NT

## *HuIgG1 wild type CH2*

ctgaactcctgggggacgctcagctctctctcccccaccccaaggacacccctcatgatctccgggacccc  
30 tgaggtcacatgcggtggtggagcgtgagccagaaacccctgaggtcaagtcaactggatcgtgagcggcgtggagggtgcataatg  
ccaaagacaaggccggaggagcagtaacacagcagctaccgtgtgtcagcgtctcaccgctctgcacaggactggctgaatgg  
cangggatcaagtccaagtctccacaaaggccctccagcccccacagagaaacacatctccaaagccaaa

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**HuIgG1 wild type CH2 AA**

pellggpsvlfppkpkdtlmistrevtcvvvdshedpevkfiwvydgvvhnaktkpreeqnystyrvvs  
vltvllhqdwlngkeykckvsnkalpapietkiskak

5

**NT HuIgG1 wild type CH3**

gggcagccccgagaaccacaggtgtacacccctgcccccatcccgggaggagatgaccaagaaccaggtcagcc  
tgacctgccttgctcaaggcttctatcccagcgacatcgccgtggagtgaggagacaaggcagccggagaacaactacaagaccac  
gcctcccggtgctgactcgcagcgtctcttctctatagcaagctcaccgtggacaagagcagggtggcagcagggaactctctca  
tgctccggtgagcatgaggctctgcacaaccactacacgcagaagagcctctcctgtccccgggtaaatga

10

**AA HuIgG1 wild type CH3**

gqprepqvytlppsreemtknqvsitclvkgyfypsdiavewesngqpennyktppvlidsdgsfflyskltvdk  
srwqqgnvfscvmhealhnhytqkslspspk

15

**NT HuIgG1 mutated hinge (C-C-C→S-S-S)**

galcaggagcccacaaatctctgacaaaactcacacatccccaccgtccccagca

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**AA HuIgG1 mutated hinge (C-C-C→S-S-S)**

dqepkssdkhtspspspa

25

Mutant hinge, but wild type CH2 and CH3—reads from the hinge+Ig tail,  
HIgG1MTH WTCH2CH3:

tgatcccccaaatctctgacaaaactcacatctccaccgtcctcagcacctgaactcctgggtggacgcgtcagt  
cttctcttcccccaaaaccgaaggacacctcatgatctccggaccctgaggtcacatgcgtggtggacgtgagccacgaaga  
ccttgagggtcaagttcaactgctacgtggacggcggtggaggtgcalaatgccaagacaaagccgcggggaggagcagtacaacagcacg  
taccgtgtgtgcagcgtcctaccgtcctgcaccaggactggtgaatgcaaggagfacaaggtcgaaggtctccaacaagaagcctctcca  
gccccatcgagaanaaactccaaaggcaaggcagccccggaagccacaggtgtacacccctgccccatccccggatgagctga  
ccaagaaccaggtgcagcctgacctgctggtcaaaaggcttctatccacagcagatcgccgtggagtgaggagcaatgggcagccggga  
gaacaactacaagaccagcctcccgctgtggactccgacggctcttctctctacgaagctcaccgtggacaaagagcaggtggca

30

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gcaggggaacgtctctcatgctcgtgatgcatgaggctctgcacaaccacacacagcagaagagcctcctctctcctcggglaaatgat  
aatctaga

Protein sequence: Mutant hinge, but wild type CH2 and CH3

5 dhpksdkthtspssapellggpsvflfpkpdkdtlmisrtevtcvvvdvshodpcvkfnwyvdgvevhna  
lktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlppsdeltknqvsitclvkgyfypsd  
iavewesngpennyyktpplvdsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispk

LLG1-5'bg1 35 mer Llama IgG1 5'  
10 5'-gtt gtt gat caa gaa cca cat gga gga tgc aag tg-3'

LLG2-5'bg1 32 mer, Llama IgG2-5'  
5'-gtt gtt gat caa gaa ccc aag aca cca aaa cc-3'

15 LLG3-5'bg1 33 mer, Llama IgG3-5'  
5'-gtt gtt gat caa ggc cac cac agc gaa gac cc-3'

LLseqsense 19mer, llama sequencing primer  
5'-ctg aga tog agt tca got g-3'

20 LLseqAS 19 mer  
5'-oct cct ttg get tig tet c-3'

NT  
25 2H7 scFv llama IgG1

aagcttgccgcacatggattttcaagtcgacattttcagcttcctgtaactcagtgcttcagtcataattgccagaggaca  
aattgtctctccagcttcacagcaatcctgtctgcatctccaggggagaaaggtcacatgacttcagggccagctcaagtgtaagtacat  
gcactggtaccagcagaagccaggtatcctccccaaacccctggatttatgcccatcaaacctggctctcggagtccctgctgctgcttcagtg  
gcagtggtgtgggacotcttactctctcacaatcagcagagtgaggctgaagatgctgccaatttactgccagcagtgaggtttaacc  
30 caccacggttcgctgctgggacaaagctggagctgaagatggcggtgctcggcggtggtggtgctgagggaggtgggagctctca  
ggcttatctacagcagctctgggctgagctggtgagggcctgggctcagtggaagatgtcctgcaaggctctggctacacatttaccagtt  
acaatgatgcactgggtaagcagacacctagacagggcctggaatgattggagctattatccaggaatggtgatactctcacaatcag  
aagttcaaggcgaaggccacatgctgtagacaatactccagcagacgctcatgacagtcagcagcgtcagctcatctgaagactctgcg

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gtctatttctgtgcaagatggtgactatagtaacttactgtacttcgatgtctgggacacaggaccaggcaccgtctctctgatca  
 aagaaccacatggagatgcacgagccccaagtgccccaatgcccgcacnccnccgaactccagaggccctctgtcttctctccccc  
 gaaacccaaggagctctctccatttttgaggccagtcacgtgctgtgtagtgagctgcggaaagaaagaccccagggtcaattcaac  
 tgggtattatggctgttgagggtgcgaacggccaalacgaagccaaagagggaacagltcaacagcacgtaccgctggtcagcgtctc  
 5 cccatccagcaccaggactgctgagcgggaaaggaattcaagtcgaaggtcaacaacaagctctcccggcccccacagagggacca  
 tctccaaaggccaaagggcagaccgggagccgaggtgtacacctggcccccacacggggaagaactgcgcaaggacacgtgagc  
 gtaacatgctctgtcaaaagcttctaccagctgacatcaactgtagtggcagaggaacgtgcagccggagctcagaggccactacgc  
 caacacgcccgcacagctggacacgacgggacctacttctctacagcaagctctcgtgggaaaagaacgtggcagcggggaga  
 aaccttaactctgtggtgtagtcatgaggccctgcacaacactacaccagaatacctaccagctctcgggtaaatagtaactaga  
 10

#### AA 2H7 scFv llama IgG1

mdfvqvfifllisasviiarqivlsqspailsaspgkvmtcrasssvsymhwyqqkpgssklpwyapn  
 lasgvparfsgsgtsylstrveadaatyccqqwsfnptfgagtkleldkgsgsgsgsgsgsgsqylqqsgaelvrpgasvk  
 15 msckasgytfsynmhvkwktpqrglewigaipyngdtsynqkfkglkatlvdksstaymqllsslsedsavfcarvvyysn  
 ywyfdwvgtgtvtvsddqephgctcpqcpapelpggspvfvfpkpkdvlsifgrvtcvvvdvgkkdpvnfnwiydigeve  
 rtantkpkceeqfnstyrvvsvlpqhqdwlgtkefkckvnnkalpapiertiskakgqtrepqvytlaphreelaktvsvtlkvgyfp  
 adlinvewqmgpeseqgyanppqlidndgtyflysklsvgkntwqrgetlcvnmhealnhytqksitqsgk

20

#### NT 2H7 scFv llama IgG2

aagcttgccgccatggatttcaagtcagatttccagcttctgtaactcagctctcagtcataattgccagaggaca  
 aattgtctctcccagctccagcaatctgtctgcatctcaggaggagaaggtcacatgacttcaggccagctcaagtgtaagtacat  
 gcaactgtgtaccagcagaagccagatctccccaaacccgtgattatgccccatcaacctgctctgagctcctgtcgtcctcagtg  
 25 gcagtggtgtctggacctcttctctcacaatcagcagagtgaggctgaagatgctgcactattacttcgcagcagtggaagttaaac  
 caccacagctctgctgctgggaccaagctggagctgaaagatggcggtggtcctggcggtgtgtgactgtgaaggagtggtgagctc  
 gcttatctacagcagctctggcggtgagctggctggcggtcctcagtgaaagatgctcgaagcgttctgtcactacacatttaccagt  
 acaatatgactgggtaaagcagacaccttagacaggccctggaatggattggagctattatccaggaaatgtgatacttctataacag  
 aagttcaagggaaggccacacigactgtagacaalctccagcacagcctacatgcagctcagcagccgacatctgaagactctgcg  
 30 gctctattctgtgcaagatggtgtactatagtaacttactgtacttcgatgtctgggacacaggaccacggcaccgtctctctgatca  
 aagaaccagaagaccaaaacacaacacacaacccaacccaacccaatctacacagaatcaaggtgcccaaatgtccagccccg  
 agctctcgggagggccctcagcttctctccccgaacccaaggagcgtctctctcattctggggagggcccgaggtcacgtgctgtgtg  
 gttagacgtgggcccaggaagaccocagggtcaggttcaactgtgacattgatggcgctgaggtgcgaacggccaacacagggccaaag

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aggaacagcttcaacagcacgtaccgcgtgggtcagcgtcctgccatccagcaccaggactggctgacggggaaggaattcaagtgcna  
ggtaacaacaagctctccggcccccattcgagaagaccatctccaaggccaaaggcagacccggggagccgcaagggtgacacct  
ggcccccacaccgggaagagctggccaaggacaccgtgagcgttaacatgctggtcaaaaggctctaccaccctgatalcaacgttgagt  
ggcagagggaatggggcagccgggagtcagagggcacgtacgccaccacgccaccccgctggacaacgacggcgacctatctcttaca  
5 gcaagctctcgtgtggnaaagacacgtggcagcagggaagaaccttcacctgtgtgtgtagtcacgagccctgcacaaccactacacc  
cagaaatccatcccccagcttccgggtaaatagtaatctaga

AA

2H7 scFv llama IgG2

mdftvqifsfllisavliarqivlsqspailsaspgekvtmtcrasssvsmhwyqqkpgsspkpwiyapsn  
lasgvparfsgsgtsytsltsrveadaatyccqwsfnptfagtklelkdggsgggsgsgggssqylqsgaeivrpagsvk  
msckasgytftsynmhwkvtprqglewigaipngdtsynqlkfkgtatlvdkssstaymqllsitedsavycarvvyysns  
ywyfdvwgtgtvssdqeptkpkppqpqpnpntteskcpkcpapellggpsvfi fppkpkdvlsisgrpevtcvvdvgqed  
pevsfnwyidgaevrantprkeeqfnstyrvsvlpiqhqdwtlgefkckvnnkalpapietkiskagqtrepvytilaphreela  
15 kdtvsvtlckvkgfypndinewqrngqpesegtyattppqldndgilyfysklsvgkntwqqgetftcvvmhealnhhytqksitqss  
gk

NT

2H7 scFv llama IgG3

angctggcccatgattttcaagtgcagallttcagcttctgctaatcaagtgttcagtcagtcataattgccagaggaca  
aatgtttctcccgctctccagcaatctgtctgcatctccagggggaagggtcacatgacttgcaggccagctcaaglgtaagtacat  
gcactgtgtaccagcagaagccaggtatctccccaaacctggalltatgcccatccaacctggctctggagtcctgctgcttcagtg  
gcagtggtgtctggacctctactctctcacaatcagcagagtgaggctgagtgatctgccacttatctgcccagcagtgagtggttttaacc  
caccacgttcgtgtctggaccaagctggagctgaaagagtgccgtgtctggcgtgtgtgtgatctggagagtggtggagctcga  
25 ggtctatctacagcagctgtgggctgagctlgtgaggcctggggcctcagtgaaagtgctctgcaaggtcttctggctacatttaccagtg  
acaatalgcactgggtaagcagacacctagacaggccctggaaatggattggagctatttatccaggaaatggtgtatcttctcacaatcag  
aagttcaaggccaaggccactgactgtatgacaatctccagcagagcctacatgcagctcagcagccctgacatctgaaagactctgcg  
gtctatttctgtgcaagagtggtgtactatagtaactcttactgtgacttctgatgtctgtgggcacaggaccacggtaaccgtctcttgatca  
agcgaaccacagcgaagaccocagctccaaggttcccaatgccaggccctgaactcttggaggggcccaagggtcttcttctccccc  
30 gaaagccaaggagcttctctccataccggaaacctgaagggtcacgtgtgtgtgtggagctgggttaagaaagacctgagatcaggttc  
aagctgtgtcctgtgatgacacagaggtacacagcgtgagacaaagccaaaggaggaacagttcaacagcacgtaccgcgtgtgcagc  
gtcctgccatccagcaccaggactggctgacgggggaaggnaatcaagtgcaaggtaacaacaaggctctccagcccccattcgagag  
gacctatctcaaggccaaggggcagacccgggagccgcaggtgtacacccctggcccaacccgggaagagctggccaaggacaccg

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2H7 scFv llama IgG3

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**2H7<sub>scFv</sub> WTH WTCH2CH3**

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ccacgcctccgcgtgctgactccgacggctcctctctctacagcaagctcacccgtggacaagagcaggtggcagcaggggaacgtct  
tctcatgctccgctgatgatgagcctctgcacaaccaclacacgcagaagcctctccctgtctccgggtaaatgatctaga

2H7+Completely WT IgG tail:

5 2H7 scFv WITH WTCH2CH3

Protein sequence

mdfqvqifsfllisasviargqivlsqspailsaspgekvtmicrasssvsymhwyqqkpgsspkpwiypasn  
lasgvparfsgsgsgtsysltisrveadaatyqcqwsfnpptfgagtdelkdggsgggsgsgsgs5qaylqsgaelvrpgasvk  
msckasgytftsynmhwvkqprqglewigaiypngdtsynqkfkfgkatltvdksstaymqllsitsedsavfyfcarvvyvns  
10 ywyfdvwtgttvssdqepkscdkthtppcapellggpsvflfppkpkdtlmisrtevtcvvdvshedpevkfnwyvvdg  
evhnaktkpreeqynstyrvvslylhqdwlngkeyckckvsnkalpapiektiskakgqprepqvyltppsrdeltknqvsitclvk  
gfyfysdiawesngqpenyktppvldsdgsfllyskltvdkrswwqgnvfscvmhealhnhytqkslslspgk

NT

15 CD80 transmembrane domain and cytoplasmic tail (+restriction sites)

ggcgatccttgcgaacctgctccatcctggcgccattacctaatctcagtaaatggaaattttgtgatgctgcotgacc  
tactgctttgccccaaagtgcagagagagaaggaggaaatgagagattgagaagggaaggtgacgccctgtataaatcgat

AA

20 *CD80 transmembrane domain and cytoplasmic tail*

adpsnllpswaitisvngifvicctycfaprrerrmerlrresvrpv

NT

25 40.2.220 VL (anti-human CD40 scFv #1--VL)

aagcttatggattttcaagtcgacgattttcagcttctcgtactatcagtcgttcacgataaagtcacagagagtgacatt  
gttctgactcagtcctcagccacccgtctgtgactccagagatagatctctcttcttcagcggccagccagagattatgcgactacttac  
actggtatcaacaaaatcacatgagctccaaaggctctctcaacaaatgcttccattccatcctctggatccctccagtggaatgagtcag  
ggtacagggtcagatttactctcagatatacaacagtgtagaacctgaagatgttggaaatttactgtcaacatggttcacagcttccgtggac  
30 gttcgggtgaggaccaaagctggaatacaaacgg

AA

40.2.220 VL (anti-human CD40 scFv #1--VL)

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mdfqvqifslisavimsrgvdivltqspatlsvtpgdrvslscrasqsisdylhwyqqkshesprllikyashsi  
sgiprsfsgsgsdftlsinsvepedvgiyycqghghsfpwtfgggtklcikr

NT

5 40.2.220 VH (for anti-human CD40 scFv #1--VH)

cagatccagtttggtgcaatctggacctgagctgaagaagcctggagagacagtcagatctctgc-aagcctctg  
ggfatgccttcacaactactgggaatgcagtggtgcaagagatgccaggaaagggttgaagtggattggctggataaacacccactctg  
gagtgccaaaatagttagaagacttcaaggacggtttgccttctctttggaaaccttgcacaactgcataattacagataagcaacctcaaa  
gatgaaggacacggctacgtattctgtgtgagatccgggaatggttaactatgacctggcctactttgcttactggggccaaagggaactgggt  
10 cactgtctctgatca

AA

40.2.220 VH (for anti-human CD40 scFv #1--VH)  
qiqlvqsgpelkpgetriskasyaflitgmqvwvqempgklkwigwintplwsalcirrlqgrfafslets  
15 antaylqislnkdedatatyfcvrsngnydlafaywgcgtltvts

NT

40.2.220 scFv (anti-human CD40 scFv #1)

20 aagcttatggattttcaagtgagattttcagcttctgctaalcagtgcttcagtcataatgtccagaggagtcgacatt  
gttctgactcagtcctccagccacctgtctgtgactccaggagatagagtcctcttcttcgaggccagccagagtattagcgactactac  
actggtatcaacaaaaacacatgagctccaaggcttctcaataatgtctccattcactctctggatgccctccaggctcagtgagcgt  
ggatcagggtgcagatttactctcagtatcaaacagtgtagaacctgaagatgttggaattattactgtcaacatggtcacagctttcgtggac  
gttcgggtggaggccacaagctggaaatcaaacggggtggcgggtgctcgggcggaggtgggtgggtggcggcggatcgaagatcca  
25 gttgggtgcaatctggacctgagctgaagaagcctggagagacagtcaggaatctctcgaaggctctcggatgctcctcaacactactgga  
atgcagtggtggcgaagagatgccaggaaagggttgaagtggattggctggataaacacccactctggagtgccaaaatagtgaanga  
cttcaaggacgggtttgccttctcttggaaacctctgccaactgcataattacagataagcaacctcaaaatgaggacacggctactgtatt  
tctgtgtgagatccgggaatggttaactatgacctggcctactttgcttactggggccaagggaactgttgcactgtctctgatca

AA

30 40.2.220 scFv (anti-human CD40 scFv #1)

mdfqvqifslisavimsrgvdivltqspatlsvtpgdrvslscrasqsisdylhwyqqkshesprllikyashsi  
sgiprsfsgsgsdftlsinsvepedvgiyycqghghsfpwtfgggtklkrrggsgggsgggsgggsggiqlvqsgpelkpgetrisk

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ckasgvaftttgmqvwvqempgkglkwigwintplwsakicrlqgrfafsletsantaylqisnlkdedytfcvrsngnydlayfa  
ywgqgtlvts

NT

5 2e12 VL (with L6 VK leader peptide)

atggatttcaagtcagatttcagcttctgctaatacagtgcttcagtcataatgtccagagagtcgacattgtgctc  
accgaatctcagcttcttggctgtgtctctaggtcagagagccacatctcctgcagagccagtgaaagtgatgaatattatgcacaagttt  
aatgcagtggtaccacaagaacaggcagagccacaaactctcatctctgctgcacacagtagaactctgggtccctgccagggtt  
agtggtcagtggtgtgggacagacttcagctcagctcaacatccatctctgtggagggagatgattgcaatgtattctgtcagcaagaataggaa  
10 ggttctctggacgttgggtggagggcaccaagctggaantcaaacgg

AA

2e12 VL (with L6 VK leader peptide)  
mdfqvqifslilisavimsrgvdivltqspaslavslgqratisrasesveyyvtslmqwyqqkpgppkllis  
15 aasnvesgvparfsgsgsgtdfslnihpveediamyfcqqrkvpwtfgggtcklekr

NT

2e12 VH (no leader peptide)  
cagggtgcagctgaaggagtcaggacctggcctgggtggcgcctccacagagcctgtccatcacatgcacccgtctca  
20 ggggtctcattaacccggtatggtgttaactgggttcgacagcctccaggaagggtctggagtggtcgtgggaatgatatgggtgatggaa  
gcacagactataaattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtctttctaaaaatgaacagctctgcaa  
actgatgacacagccagactactctgtgccagagatggttatagtaacttctactatgtatggactactgggtccaaggaacctcagtcac  
ccgtctctca(gatctg)

25 AA

2e12 VH  
vqqlkesgpglvapsqslsiictvsgfsltygvnwvrppgkglewlgmiwgdgstdynsalkrslsitkdns  
ksqvfikmnsqtdtdaryycardgysnfhyvmdywgqgtsvtvss

30 NT

2e12scFv(+Restriction sites)  
aagcttatggattttcaagtcagatttcagcttctgctaatacagtgcttcagtcataatgtccagagagtcgacatt  
gtgctcaccgaatctccagctcttggctgtgtctctaggtcagagagccacatctcctgcagagccagtgaaagtggtgaatattatgtca

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caagtttaatgcagtggtaccacagaaccaggacagccaccacaaactctctctgctgcatccaacgtagnatctggggctccctgc  
caggtttagtgcagtggggtctgggacagctcagccctcaacatccatctgtggaggaggtgatattgcaatgtatttctgtcagcaag  
taggaaggtctctggagcttgggtggaggcaccaaagctggaaatcaaacgggtggcggctcggcgagggtgggtcgggtgc  
ggcggatctcaggtgcagctgaaggagtcaggacctggcctggctggcgccctcacagagcctgtccatcacatgcaccgtctcagggttc  
5 tcattaaccggctatgtgtgtaactgggttcgccagccctcaggaaagggtctggagtggtctgggaatgatgggtgatggaagcaca  
gactataatcagctctcaaatccagactgagcatcaccaaggacaaactccaagccaaagtgttcttaaaatgaacgtctgcaaacgtat  
gacacgccagatactactgtgccagagatgggtatagtaactttcattactatgttatgactactgggtcaaggaaactcagtcaccgtct  
cctct(gatcag)

10 AA  
2e12scFv

mdfqvqifsfllisavmsrgvdivltqspaslavslgqratiscrasesveyyvtlsmqwyqkpgppkllis  
aasnvesgvparsfsgsgtdfslnihpveediamyfcqqsrkvpwtfgggtkleikrgggsgsgsgsgsgsqvqlkespgglva  
psqslsitctvsgfsltygvnwvrppgkglewlgmiwgdgtdynsalksrslitkdnksqvfkmnslqtdtdaryycardgys  
15 nfhyvymdywgqgstvtvss

10A8 is anti-CD152 (CTLA-4)

10A8 VL (with L6 VK leader peptide)

atggatttcaagtcagattttcagcttctgctaactcagtgctcagtcataatgtccagaggagtcgacatccagatg  
20 acacagctccatctcactgtctgtcatctctgggaggcaaaagtcaccatcacttgcaaggcnaagccaagcattaagaagtatataggttg  
gtaccaacacaaagcctggaaagggtccaggctgctcatattacacatctacattacagccaggcatccatcaagggttcagtggaagt  
ggctctgggagagattattccctcagcatcagaaacctggagcctgaagatattgcaactntattgtcaacagatgataatctccattgacg  
ttcggctcggsgacaaagttggaataaaacgg

25 AA  
10A8 VL

mdfqvqifsfllisavmsrgvdiqmtqspsslsaslggkvitckasqdikkyigwyqhkpqgprlllyyst  
lqpqipsrfsfgsgsgrdylsirmlepediatyyccqydnplptfsggtkleikr

30 NT  
10A8 VH ( no leader peptide)

gatgtacagcttcaggagtcaggacctggcctcgtgaacattctcagtcctctctcaccctgctctgtcactgagta  
ctccatcaccagtggtttctactggaactggatccgacagttccgggaacaaactggaatggatggccacataagccacgacggtagg

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aataactacaaccatctctcataaactgaatctccatctcgtgacacatctaaaccagttttcttgaagttagttctgtgactactgag  
gacacagctacatatcttctgtgcaagacacacacggtagtagcggagctatggactactggggccaaggaaacctcagtcaccgtctcctctga  
tca

5 AA

*10A8 VII*

dvqlqesgpglvkpsqslstcsvtgysitgfywnwirqfpgnklewmgshishdgrnnynpslinrisitrdtsk  
nqflklsstvttdatfyfcarhygssgamydgwqgtsvtvss

10 NT

*10A8 scFv*

aagcttatgattttcaagtcagattttcagcttctgctaactcagtccttcagtcataatgtccagaggagtcgacatc  
cagatgacacagtcctccatctcactgctgcatctctgggagcgaagtaccatcactgcaaggcaagccaagacattaaagaagtatat  
agggttggtaccacaacaaagctggaaaaggctccaggtgctcatatattacacatctacattacagccagcattcccatcaagggttcagtg  
gaagtgggtctgggagagattatccctcagcatcagaacctggagcctgaagatatgcaactattattgtcaacagtatgataatcttc  
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tctcataaatcgaatctccatcactcgtgacacatctaagaaccagttttcctgaagttgagttctgtagtactaggacacagctacataatt  
ctgtgcaagacactacgtagtagcggagctatggactactggggccaaggaaacctcagtcaccgtctcctctgatca

20 AA

*10A8 scFv*

mdfqvqifslisavimsrgvdiqmtspsslsagsgkvititckasqdkkyigwyqhkpgkqprliiyyst  
lqpgiprsfsgsgsgrdylsimlepediatyqqydnlpitfsgtkleikrgggsgggsgggsgsdvqlqesgpglvkpsqslst  
csvgysitgfywnwirqfpgnklewmgshishdgrnnynpslinrisitrdtsknqflklsstvttdatfyfcarhygssgamydgw  
qgtsvtvssd

30 NT

40.2.220-hmtIgG1-hCD80

aagcttatgattttcaagtcagattttcagcttctgctaactcagtccttcagtcataatgtccagaggagtcgacatt  
gttctgactcagtcctcagccacccgtgctgtagtccaggagataggtctctcttctcagggccagccagagatttagcgactatcac

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5 atgcagtgggtgcaagagatgcagagaaagggtttgaagtggatggctgataaacccccactctggagtgcacaaatagtgaaga  
cttcaaggagcgggtttgctctcttttgaaaacctctgcacaactgcatatfflacagataaggcaacctcaaaagatgaggacacggctacgtatt  
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10 gtggacggcgtggaggtgcatatagccaaagcaaaagccggggagggagcagtacaacgacgtaccgtgtgtgacgtctccaccg  
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15 gtgatgcatgaggtctctgacaaaccactacacgacgaagagcctctcctgtctccgggtgaagcggatctctgaaactctgctccacatct  
ggggcattacctatctcagtgaatgtgatatgctgctgctgacactgcttggccccaaagatgcagagagagagaaagggaagaatg  
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AA

20 40.2.220-hmtIgG1-hCD80

mfdqviqifllisavimsrgvdiivltqspatlsvtpgdrvslsrasqsisdyllhwyqqkshesprllikyashsi  
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25 kpreeqnystyrsvslvlhqdvlngkeykclksvsnkalpapicktiskakgqprepqvlytppsrclctknqslctlvkgfypsadia  
vewesngapennyktpvldsgsfllysklvtlksrwqqgnvfscvmhealnhhytqlslspgkadpsnlpawaitisvn  
gfviccltycfaprcerrrrnerlrsrvp

NT

30 2eI2seFv- hmtIgG1-CD80 fusion protein

aagcttatggatttcaagtgacgaatttcaagcttctgctaatcagtgcttcagtcataatgtccagagagagtcgacatt  
gtgctcacccaatctccagctcttctgtgtgtctctgagtcagagacacatctcctgagagccagtggaagtgttgaattatgtca  
caagtttaatgcagtgtagtcaacagaacagagacagccacccaactcctatctgtgcatccaactgagaatctgggtccctgc

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gaagtgggtctgggagagattatccctcagcatcagaacctggagcctgaagatatgcaacttatttgaacagtatgataatcttc  
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5 tctcataatccgaaltccalcactcgtgacacatctaagaccagttttctgaagttgagttctgtgactactgagacacagctacatat  
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15 ttaccttaactcagtaaatggattttgtatgtgctgacctgactgttggcccaagatgcagagagagaaagggaatgagagatt  
gagaaegggaagtgtacgccctgtataaatcgat

AA

*10A8 scFv-hmIgG1-CD80*

20 mdfqvqifslisavmsrvgvdiqmtqspsslsaslggkvitckasqdikkyigwyqhkpgkplrliiytst  
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csvtgysitsgyfwnwrfqpgnklemwghishdgrnynpslnrisitrdtsknqfllkssvttediatyfcarihyssgamdwyw  
qgtsvtssdlepksdkthtspspapellggssvflfppkpkdmlisrtpetvvdvshdepvfkfwnwydgvvhnaktkp  
reeqynstyrsvsvltvlhqdwlngkeykckvsnkalpapiekiiskakgqprepvytppsdeltknqvsitclvkgyfpsiave  
25 wesngqpennyktppvldsdgsfflyskltvdksrwqgnvfscvmhealnhhytqslslspgkadpsnllpswaitisvngf  
vicctyctfaprcrrmerlresvrv

NT

*500A2-hmIgG1-CD80*

30 atgtgtatacatctcagctccttgggctttactctcttggafttcagcctocagangtgcatagtgctgactcagctc  
cagccactctctctaatctctgggaaagatcacaatgacctgtgaagaccagtcagaataatggcacaattctacagctgtatcaccaaa  
aaccaaaagggtgctocanaggctctcatcagatgtctgcagctccatctctgggatcccccacagatcagcagtggtcggnaaca  
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5 ggcgtgtagcgacgggcccgtctatggtactactggggtcaggggatcgaagtaccgtctcctctgactctggagcccaaatctctgcanaa  
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10 agccccgagaaccacaggtgtacaccctgccccatccccgggatgagctgaccaagaaccagggtcagcctgacctgctgctcaagg  
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15 aaagtgtacgccctgtataaatcgt

AA

*500A2-hmtfgG1-CD80*

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20 srfsgsgsetdfisinnlepddgiyyccqsrswpvtfgpgtkleikrgggsgggsgggsgqvkllqsgselgkpgasvklseks  
gyiftthyiswvkqkpgesqlwignvygnggtsynqkfqgkatltvdksistaymelssltedsaiyyccarrpvatghamdywg  
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wesngapennyktppvldsdgsfflyskltvdkrsrqgnvfscsvmhleahhytqklsispgkadpsnllpsswaitlisvngif  
25 viccltycfaprcrerrnmerlresrvp

NT

*2H7 scFv MTH(SSS)WTCH2CH3*

aagctggcgccatggattttcaagtcagatattcagcttctgctaatcagtgcttcagtcataaattgccaggcgaca  
aattgtctctccagctccagcaatcctgtctgcatctccaggagaaaggtcacaaatgacttgcaggccagctcgaagtgaagtacat  
gcactggtaccagcagaagcagatcctccccnaacctggatttatgccatccaacctgctctcggagccctgctcgtcactgag  
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30

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15 2H7 scFv MTH(SSS)WTH2CH3 protein sequence:

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lasgvparfsgsgtsysltisrveacdaatyycqwsfnptfgagtkleldggsgggsgggsggssqaylqsgaelvrpgasvk  
msckasgyftsynmhwkvktpqrglewigaiypngdtsynqkfkgtatitvdksstaymqllsitsedsavyfcarvvysns  
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20 evhnactkpreeqynstnyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepvytippsrdeltknqvsitclvk  
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HuIGMHncs1 (oligo for CSS)

gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac

25 HuIGMHncs2 (oligo for SCS=ncs2)

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tgc cca ccg

HuIGMHncs3 (oligo for SSC=ncs3)

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tgc cca cca cct g

30

hIgWT3xba (3' oligo for above mutation introduction)

gtt gtt tct aga tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g

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Vhsr11: (oligo for Leu to Ser at VH11)

gga ggt ggg agc tct cag gct tat cta cag cag tct ggg gct gag tgg gtg agg cc

huIgG1-3' (3' oligo to amplify IgG1 C regions, 3' end of CH3)

5 gtc tct aga cta tca ttt acc cgg aga cag

huIgA/Gchim5 (oligo for pcr#1)

cca tct ccc tca act cca cct acc cca tct ccc tca tgc gca cct gaa ctc ctg

10 huIgAhg-5' (oligo for pcr#2)

gtt gtt gat cag cca gtt ccc tca act cca cct acc cca tct ccc caa ct

huIgA3'

ggt gtt tct aga tta tca gta gca ggt gcc gtc cac ctc cgc cat gac aac

15

2H7 scFv IgAH IGG WT CH2CH3, 2H7 scFv with IgA hinge and WT CH2 and CH3

20 aagcttgccgccatggatttcaagtcagattttcagcttcctgctaatacagtgcttcagtcataattgccagaggaca  
aatgtttcttccagctctccagcaatcctgtctgcatctccaggggagaaaggtcacaatgacttcaggcccaagcgaagtgtaagtlacat  
gcactgggtaccagcagaagccaggatctctcccaaaccttgatttatgcccatccaacctggcttctggagtcctctgtctgttcagtg  
gcagtggtgtctgggaaccttactctctcacaatcagcagagtggaagctgaagatgctgccactttactgcagcagtgagggttttaacc  
caccnagctgggtgtctgggaccaagctggagctgaagatggcgggtggctcggcgggtgtgtgatctggaggaggtgggagctctca  
ggcttattctacagcagcttgaggctgagctgtgaggcctggggcctcagtggaagatgctcgcgaaggtcttctgtacacattaccagtt  
25 acaatatgcactgggttaagcagacacctagacagggcctggaatggatggagctattttatccaggaatgtgtatcttcciaaatcag  
aagttcaaggggcaagccactgactgtagacaatctctcagcagccctacatgcagctcagcagcctgacatctgaagactctgcg  
gtctatttctgtcgaagagtggtgtactatagtaacttactgtgacttcgaltctggggcaacaggaccagggtaccgtctctgacagc  
caggttcctcaactccaccctacccatctccctcaactccaccctacccatctccctcatgctgcacactgaaactcctggggggaccgtagtct  
tctcttctcccccnaaaccaaggacacccctcatgatctccggaccctgaggtcacatgctgtgtgtggagcgtgagccacgaagacc  
30 ctgaggtcaagttcaactgctacgtggagcgcgtggaggtgtgcatatgtccaaagacaagccggggaggaagcgtacataacagcacgla  
ccgtgtgtcagcgtctcaccgtctgtcaccaggagctggctgaatgtgcaaggaggtacaaggtcaaggtctccacnaaagccctccagc  
cccatcgaagaanaactctccaaagccaaagggcagccccgaggaaccacaggtgtacacctgtcccccattccgggatgagctgacc  
aagaaccaggctcagctgacctgctgtgtcaaaaggcttctatccagcagcatcgccgtggagtgggagagcaatgggcagccggaga

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acaaactaagaccacgcctcccgtgctggactccgacgctccttctctacagcaagctaccgtggaacagacagcggggcagc  
aggggacgctctcatgctccgctgatgatgagcctctgcacaaccactacacgcagaagagcctcctctgctccgggtaaatgatct  
aga

5 2H7 scFv IgAH IGG WT CH2CH3 protein sequence

mdfvqqlfllisavliargqivlsqspailsapgekvtmterassvsymhwyqqkpgsspkwiyapsn  
lasgvparfsgsgstysltirveadaatyycqwsfnpptfgagtkleikdggsgggsgggssqaylqqsgaelvrpgasvk  
msckasgyftfysnmhvwkqtrpqglewigaiypngdtsynqkfkqkaltlvdksslaymqllssltedsavfyfcarvvyysns  
ywyfivwgtgtvtsdqpvpstptpsptpspcapellggpsvflfppkpkdltlmisrtpetvctvvdshdedpckfnwy  
10 vdgvevhnaktkpreeqynstyrsvslvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvyltppsrdeitknqvsit  
clvkgyfypsdiawesngqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhhytqkslslspgk

NT

2H7 scFv IgAH IgACH2CH3 (2H7 scFv IgAhinge and IgA CH2 and CH3)

aagctgcgccatggatttcaagtcagatttcaagctcctcgtatacagtccttcagtacataattgccagagagaca  
15 aattgtctctccagcttcacgaatcctgctgcatctccagggaagaagtcacaatgacttgcaggccagctcaagtgtaagtacat  
gcactggtacagcagaagccagatcctccccaacccctggatttatgcccatccaacctgctcctgagtcctgctcgttcagtg  
gcagtgaggctctgggacactcttactctctcaaatcagcagagtgaggctgaaagatgctgccactattactgccagcagtgagggttttaacc  
caccacagctcggctcggaccaaagctggagctgaagatggc-gtggctcgggcgggtggatgtggagagggggagagctcga  
ggcttatctacagcagtgctgggctgagctggtgagggcctgggacctcagtgaaagatgtcctgaaggctctggtacatacattaccagtt  
20 acaatatgcactgggtaagcagacacctagacagggcctggaatggattggagctatttatcaggaatgtgtatattctcacaatcag  
aagttcaaggggcaaggccacactgactgtagacaaatcctccagcacagcctacatgcagctcagcagcctgacatctgaagactctgcg  
gtctattctctgaagagtggtgactatagtaactcttactggtactctgatgctggggcacaggggaccaggtcaccgtctctctgatca  
ggcaggtctcccaactccacctacccatctccctcaactccacctaccctatctccctcatgctgccaccccgactgctcactgcaccgacc  
ggccctcgaaggactcgtcttaggttcaagaagcagatctcagtcacactgaccggcctgagagatgcctcagtggtgacacttcaactgg  
25 acgcccctaagtgggaagagcgtgttcaaggaccacctgaccgtgacctgtggtgctgctacagcgtgtccagtgctcgtgccgggctgt  
ggcagggcatggaacatgggaagacctcactgctgctcctaccccgagtcgaagaccccgtacacggccacccctcacaatacc  
ggnaacacatctccggccagggctcacctgctgccgccgctcggggagagctggccctgaacagagctggtgacgctgacgtgctg  
cacgtggctcagccccaagatgtgctgttcgctgctgaggggtgcacagggagctgccccggagagatgacctgacttgggacatcc  
ggcagggagccacagccaggccaccacaccttcgctgtgacagcctatcgtgcggtggcagccgagagctggaagaagggggacacct  
30 tctctgcatggtggccacgagggccctgccgctggccttcacacagaagacacatcgaccgttggcgggtgaaacccaacctatgcaatg  
tgtctgtgtcatgctggcggaggtggacggcacctgctactgataatctaga

AA

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2H7 scFv IgAH IgACH2CH3 (2H7 scFv IgA hinge and IgA CH2 and CH3)

mdfivqvifslisasviiargqivlsqspailsaspgekvtmtcrassvsymhwyqqkpgsspkpwiypasn  
lasgvparfsgsgtsysltisrveaadaatyccqwsfnptpfagtkleldggsgggsgsgssqaylqqsgaelvrpgasvk  
msckasgytfsynmhvkvtpqrqlewigaiypngdtsynqkfkkgkatlvdkssstaimqlssltedsavyfcarvvyvns  
5 ywyfdvwtgtttvssdqvpstptptpsptptpspcchprlsilhrpaedlllgseailctltglrdsagvtftwtpssgksavqgp  
pdrldcgysvssvlpgaepwnhgtfcttaaypesktpiltalsksgntfrpevhlpppseelalnelvticlargfspdvlvrwlq  
gsqelprekyltwasrqpsqgtttfvtlsilrvaedwkkgtfscmvghaelplafktidrlagkpthvnsvvmaevdgtcy

IgA hinge-CH2-CH3 (Human IgA tail, full length)

0 tgatcagccagttccctcaactcaccacccatccctcaactcaccacccatccctcatctgctgccaccc  
cgactgtcactcaccgacggccctcagggacgtctcttaggtcagaagcgtacgtcgtcagcactgacggccgtcagagatgccc  
tcagggtgacacctcaccctgagcgcctcaagtgggaagacgtctgtcaaggaccactgacccgtgactctgtgctgtctacagcgtgt  
ccagtgctctgccggcgtgtgcccagccatggaacatgggaagacgtcactgtgctgctcctaccccgagtcacagaccccgctaa  
ccgcccacctctcaaaalcggaaacacattccggccggaggtcaccgtctgccgccgctcgaggagagctggccctgaacgagct  
15 ggtgacgtcagctgctggcacgtggcttcagcccccaggaatgtgctggttcgctgctgacgggggtcacagagctgcccgcgaga  
agttacctgactgggcatcccgagagagccagccagggcaccaccactctcgtgtgaccagcactatgcgcgtggcagccgagga  
ctggaagaagggggacacctctcctgcatgggtggccacgagggccctgccgtggccttcacacagaagaccatcgaccgctggcgg  
gtaaaccaccatgtcaatgtgtctgtgcatgcggaggtggacggcactgtctactgataatctaga

IgA hinge-CH2-CH3 Protein sequence, (Human IgA tail, full length)

20 Dqpvpstptptpsptptpspcchprlsilhrpaedlllgseailctltglrdsagvtftwtpssgksavqgppdr  
dlcgysvssvlpgaepwnhgtfcttaaypesktpiltalsksgntfrpevhlpppseelalnelvticlargfspdvlvrwlqsg  
elprekyltwasrqpsqgtttfvtlsilrvaedwkkgtfscmvghaelplafktidrlagkpthvnsvvmaevdgtcy

Human J Chain:

agatctcaagaagatgaaggattgtcttctgtgacacaaatgtaagtgtgccggattacttcaggatcatccgttc  
ttccgaagatctaatgaggacattgttgagagaacatccgaattatgttctctgaacacaggagaaatctctgatccaccctacc  
attgagaaccagattgttgatccattgtctgacctcagctgtaaaaaatgtgatctacagaagtggagctggataatcatagattactgcta  
30 cccaggaacatattctgtgatgaagacagtgctacagagacctgctacactatgacagaacaagtgtcatcagactgtgtgccactcgtga  
tatgtgtgtgagacaaatgttggaacagccttaaccocagatgctgctatcctgactaatctaga

Human J Chain polypeptide

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rsqederivlvdnkckcaritsriirssedpnedivernirivplnnrenisdptslrtrfvyhlsdckkcdpsev  
eldnqivtatqsnicdedsatetcytdrmkcytavvplvyggetkmvetaltpdacyp

HUJCH5n1 (J chain 5' primer)

5 gtt gtt aga tct caa gaa gat gaa agg att gtt ctt

HUJCH3 (J chain 3' primer-antisense)

gtt gtt tct aga tta gtc agg ata gca ggc atc tgg

10 4 carboxy terminal amino acids deleted from IgA CH3  
GTCY

IgAH IgAT4 Human IgA tail, truncated (3T1)-(missing last 4 amino acids from  
carboxy terminus)

15 t g a t c a g c a g t t c c c t c a a c t c c a c t a c c c c a t c c c t c a a c t c a c c t a c c c c a t c c c t c a t g c t g c c a c c c  
c g a c t g t c a c t g c a c c g a c c g c c c t c g a g g a c c t g t c t t a g g t c a g a g c g a t c c t c a c t g c a c a c t g a c c g g c c t g a g a g a l g c c  
t c a g g t g t c a c c t t c a c c t g g a g c c c t c a a g t g g a a g a g c g c t g t t c a a g g a c c a c c t g a c c t g a c c c t g t g g c t g c t a c a g c g t g t  
c c a g t g t c t g c c g g c t g t g c c g a g c c a t g g a a c c a t g g g a a g a c c t t c a c t t g c a c t g c t g c c t a c c c g a g t c c a a g a c c c g c t a a  
c c g c c a c c c t c a a a a t c c g g a a c a c a t t c c g c c c g a g g t c c a c t g c t g c c g c c g t c g g a g g a g c t g c c c t g a a c a g a c t  
20 g g t g a c g t g a c g t g c c t g c a c g t g g c t t c a g c c c a a g g a t g t g t g t t c g t g c t g c a g g g t c a c a g a g a g c t g c c c c g c g a g a  
a g t a c c t g a c t t g g c a t c c c g g c a g g a c c c a g c c a g g g c a c c a c c a c c t t c g t g a c c a g a t a c t g c g c g t g c a g c c g a g a  
c t g g a a g a a g g g g a c a c c t t c t c t g a t g t g g c c a g a g g c c c t g c c g t g g c c t t c a c a g a a g a c c a t c g a c c g t t g c g g  
g t a a c c c a c c c a t g t a a t g t g t g t g t c a t g g c g a g g t g g a c t g a t a a t c t a g a

25 IgAH IgAT4 Protein sequence:

Dqvpvstptpsptpptspschprlsllhrpaledlllgseailtcltldrdsagvftwtpssgksavqgppdr  
dlcgcysvssvlpgeacpwnhgktftctaaypesktptlatlsksntfrpevhlpppselalnclvtlclargfspdvlvrwlqsg  
elprekyltwasrqpsqgtttfavtsilrvaaedwkkgdtfscmvghalplafitqktidrlagkpthvnvsvmaevd

30 HUIGA3T1 (Oligo 3': to delete 4 amino acids at carboxy end of IgA CH3)  
gtt gtt tct aga tta tca gtc cac etc cgc cat gac aac aga cac

HUIGA3T2: (oligo to delete 14 aa at end of IgA -T4)

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gtt gtt tct aga tta tca ttt acc cgc caa ggc gtc gat ggt ctt

NT

*2H7 scFv IgAH IgAT4*

5 (2H7 scFv IgA 3T1 construct)—truncates the CH3 domain at the 3' end

aagcttgcgcgatggaatttcaagtgcagattttcagcttcctgctaatcagtgcttcagtcataattgccaggaggaca  
aattgttctctcccagctccagcaatcctgtctgcatctccaggggagaaaggtcacaatgacttgcagggccagctcaagtgtaagttaacat  
gcactgggtaccagcagaagccagatctctcccccacccctggatttatgcccacccaacctgcttctggagtcctgtctgcttcagtg  
gcagtggtgtctggacaccttactctctcaaatcagcagagtgaggagctgaagatgctgccacttaactgccagcagtgagggttttaacc  
10 ccccacgttctggtgtgtggaccacaagctggagctgaagatggcgggtgctcgggcgggtgtgtggtatcggagaggtggagagctctca  
ggcttatctacagcagctctgggctgagctgtgaggcctggggccctcagtgaaagatgtctctcaaggcttctgtgtacacatttaccagt  
acaatafgcactgggtgaagcagacacctagacaggggcctgggaatggatlggagotattatocaggaaatggtgatactctcaaatcag  
aagttcaaggccagggccacactgactgtagacaaatcctccagcagacccatagtcagctcagcagccctgacatctgaagactctgg  
gtctatttctgttcgaagagtggtgtactatagtaacttactgggacttctgagtgctggggcagcaggaccagggcaccgtctctctctgatac  
15 gccaggttcctcaactcaccatcccccattcctcactccactcaccatccctcctcctgctgcacccccgactgtcaatcgaaccgacc  
ggccctcagagacgtctcttaggttcagaagcagatctcactgtgcacactgacccggcctgagagatgctcctcaggtgtcactcctcactgg  
acggccctcaagtgagggaagagcgtgttcnaggaccacgtgacccgtgacccctgtgctgctacagcgtgtccaggtgtcctgcccgggctgt  
gccgagcctatggaaacatgggaagacccttcaactgcaactgctgccttaccgccaggtccagaccaccgctacccctctcaaaatcc  
ggaaacacattccggcccgaggtccacgtgctgcggccgctgcggagggagctggccctgaacgagctggtgacgtgacgtgctgtg  
20 cacgtggcttcagcccaaggatgtgtgtgtgtgctgctgcaggggtcagaggagctgctcccgaggaagtacgtgactggtggtgcatccc  
ggcaggagcccgacggggcaccacaccttgcgtgtgacagcagcactactgogcgtggcagccgaggaactggaagaagggggacacct  
tctcctgcatgtgtggccacaggccctcctgcgtgctgtcactacacagaagaccatcgaccgcttgggggtaaacccaccactgtaactg  
tgtctgtgtcatggcgagggtggactgatactaga

25

AA

*2H7 scFv IgAH-T4*

mdfqvqifslflisavliargqivlsqspailsaspgekvtmtcrassvsymhwyqqkpgsspkpwiypasn  
lasgvparfsqsgsgtsysltisrveadaatyccqwsfnptfagtkleldggsgggsgsgssqaylqsgaelvrpgasvk  
msckasgytfltsynmhvkwqtpqrqglewigaitypgndtsynqkfkqkatltvdkssstaymqllsitedsavycarvvyvsn  
30 ywyfdvwtgtttvtssdqpvstptpsptptpspschprlslhpaedlllgsealtcttldrdsagvtflwtvpsgsksavqgp  
pdrldlccgysvslpgcaepwnhkgfttcaaypesktpitltsksntfrpevhlppseelalnelvtitclargfspdvlvrwlq  
gsqelprekytwsarqpsqgtttfavlslrvaedwkkgdtfscmvghaelplafkqtkidrlagkpthnvsvmmavcd

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14 amino acids deleted from IgAH-T4 (so that total of 18 amino acids deleted from wild type IgA CH3)

PTHVNVSVVMAEVD

5

IgAH IgA-T18 (Human IgA Tail truncated, 3T2)

Tgactgacgagttccctcaactccactaccctacccatctccctcaactccactccctcatgctgccccc  
ccgactgtcactgcaccgaccgcccctcaggacctgctcttaggttcagaagcgtctcactgtgcacactgaccggcctgagagatgc  
ctcaggtgtcactctcactcggacgcccctcaagtgagggaagcgtctgttcaaggaccactgaccgtgacactctgtggtgctctacagcgtg  
10 tccaggtgtcctgcggggctgtgtccgagccatgggaacatgggaagaccttcacttgcactgtgctccatcccaggtccaagaccgcccta  
accggccacccctctcaaaatccggaacacattccggcccagggtccacactgctgccggccgctcgggaaggagctggccctgaacgagct  
gggtgacgtgacgtgctcggcagcgtgtgttcagcccccaaggatgtgctgttgcgtgtgctgacgggttcacagagagctgccccgcgaga  
agtaactgacttgggcatocccgcaggaagccagccaggggcaccacacacttgcgtgtgacagcactatcgcgtgtgcaagccgagga  
ctggagagagagggggacacactctctctcatggtgtggccacagggccctgccgtggccttcacacagaagaccatcgaccgctggcggg  
15 gtaaa

IgAH IgA-T18 Protein sequence:

dqpvpstptpsptpsptpspcschprlsrlrpalcdlllgseailcttlglrdasgvltftwssgksavagppdrd  
lccgysvssvlpgcaepwnhgktftctaaypeskplitaltsksyntfrpevhlpppseelalneivltctclargfskpdvlvrwlqgsq  
20 lprekyltwasrqepsqgtttfavtsilrvaedwkkgdtdfscmvgealplafiqktdrlagk

NT

2H7 scFv IgAH IgAT18: (Human IgA Tail truncated, 3T2.)

aagctgcgcccatggattttcaagtgcagatttcaactctcgtatcactgtcttcagtcatgaataaattgcagaggaca  
aattgttctccaggtctccagcaactctgtctgcatctccaggggagagaaggtcacaatgacttcagggccagctcaagtgtaagtatcat  
gcactggtaccagcaggaagccagagctatcccccaaaccttgattatgcccatccaactggcttctggaggtccctgctcgtctcaagt  
gcagtggtgtctgggaactcttactcttcacaaticagcagagtggaaggctgaagatgctgcacattattatcgcagcagtgagagtttaacc  
caccacgttgctgtgctggaccaaagctggagctgaaagatggcgtgctcggcggtggttgatctggaggaagggtgggggctctca  
30 ggcttatctacagcagctcgtgggctgagctgtgagggcctggggcctcagtgagaagatgtctcgaaggctctggctacacatttaccagtt  
acaataigcactcgtgtaagcagacacactagacaggcctgggaatggattggagctattatccaggaatgtgatactctcacaatcag  
aagttcaagggaaggccacactgactgtagacaaalccctccagcagacgctacatgcagctcagcagcctgacatctgaaagctctgcg  
gtctattctgtgcaagagtggtgtactatagtaacttctactggtacttcgatgtctggggcacagggaaccaggtcaccgtctctctgatca



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gcacggttccctcaactccaccctaccccatctccctcaactccacccatctccctcatgctgccaccccgactgtcactgcaccgacc  
ggccctcagaggacctgctcttaggttcagaaagcagatcctcagctgcacactgaccggcctgagagatgctcaggtgtcaccttcacctgg  
acgccctcaagtgagggaagcgcgtgtcaaggaccacctgacctgtgtggtgctgctacagcggtgtcaggtgtcctggcggctgt  
gcccgaagccatggaacctgggaagaccttcacttgctgctgcttaccggagtcgaagaccccgtaaccgcccacctctcaaaatcc  
5 ggaacacattccggcccgaaggccacactgctgcccggccgctcggagggagctggccctgaacgagctgtgtagcgtgacgtgctgg  
cacgtggttcagcccaagaaagtgtgctggtcgtgctggcaggggtcacaggagctgccccgggaaggtacctgacttgggcatccc  
ggcaggagcccgacggcaccaccacctctcgtgtgaccagatactgcgcgtggcagccggagactggaagaagggggacacct  
tctctgcatggtgggcacgagccctgccgctggccttcacacagaagacctgaccgcttggcggggtaaa

0

AA:

2H7 scFv IgAH IgAT18:

mdfqvqifslilisasviiaqgqivlsqpailsaspgekvtmtrasssvsymhwyqqkpgsspkwiyapn  
lasgvparfsgsgtsylsitrvaedaatyycqwsfnppftfgagtkleldggsgggsgggssqaylqsgaelvrpagsvk  
msckasgyftfynmhwwktpqrqglewigaiypngdtsynqkfkqkalitvdksstaymqllssedsavvfcarvvyyns  
15 ywyfdvwgtgtvtssdqvpstppstppstppstpschprlsilhrpaledlllgsailectltglrdasgvtftwpsggksavqgp  
pdrldcgyssvslpgcaepwnhgktftctaaypsktpitatlslsgntfipevhlpppsealnelvitclargf/spkdvlvrwlq  
gsqelprekyltwasrqepsqglttfavtsilrvaedwkkgdftscmvghealplafktidriagk

20

CTLA-4 IgG WTH WTCH2CH3 (Human-oncoMLP-CTLA4EC-hIgGWT)

Nucleotide sequence:

gcaacctacatgatgggggaatgagttgaccttcctagatgattccatctgcacgggcacctccagtggaatcaagt  
aacctcaactccaaggactgagggccatggagacgggagctcactcgaagggtgagctcatgtaccacgcacatactacctgggc  
ataggcaacggaaccagatttatgtatgatccagaaccgtgccagattctgatcaaccacaaactgtgacaaaactcacacatgccca  
25 ccgtgccagcaactgaactctgggggacggcagctcttctctcccccacaaacccaaggaacacctcatgtatctccggaccctg  
aggctacatgctggtgtggtgagcgtgagccacgaagacctgaagggtcaagtcaactgtacgtggcagcggcgtggaggtgataatgcc  
aagacaaagccgcgggaggaagcgtacaacagcacgtaccgtgtgtgacagcgtctaccgtctgcaccaggaactggtgtaaggga  
aggagtgtaagtgcaaggtctcnacaaagccctccagcccccacatcgagaacaatctccaaagccaaggcagcccccgaagc  
acaaggtgtacacctgccccatccgggagctgacccaagaacaggctcagcctgacctgctgctcaaaaggcttctatccagcga  
30 catcgccgtggagtgaggagagcaatggcgagccgggaagaacactacaagaccagcctcccgctgctgactccgacggctcctcttcc  
tctacagcaagctcaccgtggacaaagcagaggtggcagcgggaacgtcttctcatgctccgtgatcatgagcgtctgcacaaacct  
acaacgcaagaagcgtctcctctgctcctgggtaaatga

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CTLA-4 IgG WITH WTCH2CH3 Protein sequence:

mgvlltqrllslvlllfpmsmamhvaqpavvlssrgiasfvceyaspgkatevrvtlrqadsqvtvcaaa  
tymmgneiftlddsictgtssgnqvnltiqglramdtglyickvelmypyppylgigngtqiyvidpepcpdsdpkscdkthtccpc  
papellggpsvflfppkpkdtlmisrtpevtcvvvdshedpevkfnwyvdgvevhnaktkpreeqynstyrsvsvltvlhqdwl  
5 gkeykckvsnkalpapielktiskakgqprepqvylppsrdeitknqvsitclvkgfypsdiavewesngqpennyktppvlidsdgs  
fflyskltvdksrwqqgnvfscsvmhleahhnytklsislspgk

Human OncoM leader Peptide+CTLA4 EC (BcII)

Atgggggtactgctcacacagaggacgctgctcagctgtgtccttgccactcctgtttcgaagcatggcgaagcatggc  
0 aatgcacgtggccacgctctgtgtgactggtccagcagccgagcgcacgtccagctttgtgtgagtatgcatctccaggcaangccact  
gaggtccgggtgacagtgtctcgacaggtgacagccaggtgactgaagctgtgcggcaacctacatgatgggaatgattgaccttc  
ctagatgattccatctgcacggcgacctccagtggaatacaagtgaaacctcattccaaaggactggggccatggacacgggactctaca  
tctcgaaggtggagctcatgtacccaccgccatactacctggcgataggcaacggaaaccagatttatgtaattgatccagancctgtgcc  
agattctgatcaa  
5

Human OncoM leader Peptide+CTLA4 EC Peptide sequence:

mgvlltqrllslvlllfpmsmamhvaqpavvlssrgiasfvceyaspgkatevrvtlrqadsqvtvcaaa  
tymmgneiftlddsictgtssgnqvnltiqglramdtglyickvelmypyppylgigngtqiyvidpepcpdsdpk

Human OncoM leader peptide nucleotide

atgggggtactgctcacacagaggacgctgctcagctgtgtccttgccactcctgtttcgaagcatggcgaagcatg

Human OncoM leader peptide sequence:

Mgvlltqrllslvlllfpms

25

NT

Human CTLA4 EC (no LP)

Gcaatgcacgtggccacgctgctgtggtactggccagcagccgagcgcacgtccagctttgtgtgagtatgcat  
ctccaggcaaaagccactgaggtccgggtgacagtgtctggcaggtgacagccaggtgactgaagctgtgcggcaacctacatgacg  
30 gggaatgattgaccttctgattgattcatctgcacggcgacctccagtggaatacaagtgaaacctcattccaaaggactgaggccat  
ggacacgggactctacatctgcaaggtggagctcatgtacccaccgccatactacctggcgataggcaacggaaaccagatttatgtaatt  
gatccagaaccgtgccagattct

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AA

Human CTLA4 EC (no LP)

Amhvaqpavvlassrgiasfveyaspkatevrvvtrqadsqvteveaatymtgneltfddsiectgtssng  
vnltiqglramdtglyickvelmypppyylgigngtqiyvidpepcpds

5

NT

Human CTLA4 IgG MTH (SSS) MTCH2CH3

Atgggggtactgctcacagaggacgctgctcagctggtcttgcactctgtttccaagcatggcgagcatggc  
aatgcacgtggccacgctgctgtgtactggtccagcagccgagcagcagcttctgtgtgagatgcatctccagcnaagccact  
10 gaggtccgggtgacagctgttcggcagctgacagccaaggtgactgaagctctgtgcggcaactcatatgatgggaatgagttgaccttc  
ctagatgatccatctgcacgggacacctcagtggaatacaagtgaaacctactatccaaggactgagggccatggacacgggactctaca  
tctgcaaggtggagctcatgtacccaccgccatactacgtggcagcgaacggacccagatttatgtaattatccagacccgtgcc  
agattctgatcaacccaattcttgacnaaactcacacatccccaccgtccccagcaactgaactcctgggggagtcagctctctctt  
cccccaaaacccaaggacacacctcatgatctccggacccctgaggctacatgctgtgtgtgtgagctgagccacgaagacctgag  
15 gtcaagttcaactgtacgtggagcggcgtggaggtgcataatgccaagacaaagccgaggagcagtaacacagcagctaccgtg  
tggtcagctgctcaccgtctcaccaggaactggctgaatggcaaggagtaacaagtgcaaggtctccaacnaagcctccagccccc  
atcgagaanaacnattctccaagccaaggcagccccgagaccacaggtgtgacacctgcccccatcccgggagtgagctgaccaaga  
accaggtcagcctgacctgctgtgtcaaaagcttctatccagcagacatgcctgtggagtgaggagagcaatgggagccggagagaaca  
ctacaagaccacgcctcccgctgctggactccgagcgtctctctacagcaagctcaccgtggacaaagcaggtgagcagcagggg  
20 gaacgtctctcatgctcctggtgatgaggtcctgcacaaccactacacgcagaaagacgtctctcctgctccgggtaaatga

AA

Human CTLA4 IgG MTH (SSS) MTCH2CH3

Mgvlltqrlslslvllfpmasmamhvaqpavvlassrgiasfveyaspkatevrvvtrqadsqvtevea  
25 atymmgneftfddsiectgtssngqvnltiqglramdtglyickvelmypppyylgigngtqiyvidpepcpdsdpkssdkhtspp  
spapellgssvflfpkpkdtlmsrtpevicvvvdshedpevkfnwvdgvevhnaktpreqynstvrsvvltvlhqdwn  
gkeyckcvsknalpapektiskakgqprepqvylppsrdeltnqvsitclvkgfyfysdiaewesngqpenntktpvldsdgs  
fflyskltvdksrwqggnvfscsvmhlnhytkslslspgk

30

CTLA-4 IgAH IgACH2CH3 (Human-oncoMLP-CTLA4EC-IgA)

Nucleotide sequence:

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atgggggtactgctcacacagaggagcgtgctcagctggctcctcactcctgtttcaagcatggcggagcatggc  
aatgacagctggcccgagcctgctgtgtgtactgtgcccagcagccgaggcagcagccagctttgtgtgagatgacatctccaggcaagccact  
gaggtccgggtgacagctgcttcggcagcgtgacagccaggtgactgaagctgtgtgcccgaacctacatgaggggaatgagttgaccttc  
ctagatgattccatctgcacgggcacctccagtggaatcaagtgaacctcactatccaaggactgagggccatgagacacgggactctaca  
5 tctgcaagggtggagctcatgtaaccaccgccatactacctgggcataggcaacgggaaccagattatgtaattgacccaagaccgtggcc  
agattctgatcagccagttccctcaactcacctaccccatctccctcaactccactaccccatctccctcatgctgccaccccgactgtca  
ctgcaccgaccggccctcagggacctgctcttaggttcagaagcgatcctcagctgacacgtgacccggctgagagatgctcaggtgtc  
accttcacctggagccctcaagtgagggaagcgctgttcaaggaccactgaccgtgacctctgtggctgctacagcgtgtccaggtgtc  
tgcgggctgtggcggccatggaaccaatgggaagaccttactgtcactgctgctcctaccggagctcaagaccgccgctaaccggcacc  
0 ctctcaaatccggaaacacattccggccgaggtccacctgctgcccggcgtcggagagctggccctgaacgagctgtgagcgt  
gacgtgctgtggcagctggcttcagccccaaggatgtgctgttgcctgctgcaggggtcacaggagctgccccgcggaagatctga  
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gggggacacctctcctcatggtggggccacgagggccctgcccgtgctgcttcacacagaagaccatgaccgcttggcgggttaaaccca  
cccattgcaatgtgtctgtcatggcgagggtggacggcacctgctactataatctaga

5

CTLA-4 IgAH IgACH2CH3 Protein sequence:

mgvlltqrllslvlallfpmasmamhvaqpavvllasrgiasfvcyspgkagtevrvtlvrqadsqvtevcaa  
tyrmngneltfddisictgssgnqvnltiqglramdtglyickvelmypppyylgigngtiqiyvidpepcpsdqpvpstptpspt  
pptpspcschprlsihrpaledlllgseailtcltglrdasgvtflwpsgksavqgppdrldlcygssvslpagaepwnhkgkftcta  
20 aypeskipltatlsksgntfpevhllppseelalnelvtitclargfspkdvlvrwlqgsqelprekyltwasrpepsqgtttfvatsilrva  
acclwkkgtfscmvghaelplafktidrlagkpthvnsvvmaevdgtcy

CTLA-4 IgAH IgA-T4 (Human-oncoMLP-CTLA4EC-IgA3T1)

Nucleotide sequence:

atgggggtactgctcacacagaggagcgtgctcagctgtgctcctcactcctgtttcaagcatggcggagcatggc  
aatgacagctggcccgagcctgctgtgtgtactgtgcccagcagccgaggcagcagccagctttgtgtgtgagatgacatctccaggcaagccact  
gaggtccgggtgacagctgcttcggcagggctgacagccaggtgactgaagctgtgtgcccgaacctacatgaggggaatgagttgaccttc  
ctagatgattccatctgcacgggcacctccaagtgaatcaatgaacctcactatccaaggactgagggccatgagacacgggactctaca  
tctgcaagggtggagctcatgtaaccaccgccatactacctgggcataggcaacgggaaccagattatgtaattgacccaagaccgtggcc  
30 agattctgatcagccagttccctcaactcacctaccccatctccctcaactccactaccccatctccctcatgctgccaccccgactgtca  
ctgcaccgaccggccctcagggacctgctcttaggttcagaagcgatcctcagctgacacatgaccggcctgagagatgctcaggtgtc  
accttcacctggagccctcaagtgagggaagcgctgttcaaggaccacctgaccgtgacctgtgtgctgctacagcgtgtcactgctc  
tgcgggctgtggcggccatggaaccaatgggaagaccttactgtcactgctgctcctaccggagtcgaagaccgccgctaacccgcacc

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5

Mgvltftrqltllsvallfpnsasnamhvaqpavllarsgiafvcyeaspkgatevrvtrlraqdsqvtveca  
ldsdicgtssgnqvntiqglramdftlyickvelmypyppylgigntqiyvidpepcpsdqvpstptpsps  
lsllhrpaledlllgseailcttglrdaasgvtftwpsgsksavqppdrldgcysvssvlpgcapewnhgkftcta  
ksnqntfpevhllpppselaenlvttdlartgspkdvrvrwlqsgselprekyltwasrgsqgtttfvaillra  
mvghealpfatktactldagktpnvnsvmaevd

## 15

ccctgaactctctgggggggatcgtcagttcttctctcccccacaaaccaaagacacctcatgatctccggacacct  
gagggtcacatgctggtggtgctgagcgtgagccacgaagacctgagggtcaagttcaactgtgacgttgacggcgtgga  
caagacaaaccggcgggaggagcagtacaacagcagctaccgtgttgtagctctaccgtctgcacaggagctgctggaatg  
aaggaggtacaaggtgcgaaggtctccacaagaagccctccagcccccacgagaaaacatcctccaaaagccaaag

pellggssvflfpkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvs  
vltvlhqdwlngkeykckvsnkalpapiektiskak

## PAPELLGGPS

## 30

PAPELGGSS

## ggt ggt gat cac gtc tgc tcc agg gac ttc acc cc

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gtt gtt tct aga tta act ttt acc ggg att tac aga cac cgc tcg ctg g

gtt gtt ttc gaa gga tcc gct tta ccg gga ttt aca gac acc gct cgc tgg

NT

[illegible]

25

human IgE Fc (CH2-CH3-CH4) ORF:

148

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*IFhIgGwtBcl5*

gtt gtt tga tca gga gcc caa atc ttg tga caa aac tca cac atg ccc acc gtg ccc agc acc  
(63 mer)

5

hIgGWT3xba  
gtt gtt tot aga tca ttt acc cgg aga cag gga gag gct cti ctg cgt gta g

*HuIgGMHWC (sense, 5' primer for mutating wild type hinge CCC to mutant*

10 SSS'

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca cgg tcc cca gca cct gaa  
ctc ctg ggt gga cgg tca gtc ttc c

NT

15

1D8 VH

caggctgagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctccatgcactgtctctg  
gggtctcattaccagcagtggtgtacactggatcagacagcctccaggaaagggtctggaatggatgggaataatattattatgatggc  
acaagattataattcagcaattaatccagactgagcatcagcaggcgcacacctccaaggccaagttttctnaaaatcaacagctgtcgaact  
gatgacacagccatgtattactgtgccagaatccacttgattactggggccaaggagtcagtggtcacagctctcctt

20

AA

1D8 VH (no leader)

qvqlkeagpglvqptqtllstctvsgfsltsdgvhwirppgkglewmgiyydggtdynsaiksrslsrdtsks  
qvflkinslqtdtdamyycarihfdywgqvmvtvss

25

NT

1D8 VL (no leader)

gacattgtgctcactcagctctocacaacacatagctgcatctccaggggagaggtccacatcacctgcccgtgccag  
ctccagtgtaagtacatgtactgtgtaccagcagaagtcaggcgcctccctaaactcttggaattatgacacatccaaagctgcttctggagtt  
30 ccaaatgcttcagtggcagtggtctgggacctctattctctcgcaatcaacacatgggactgaagalgtgccattattactgtcagc  
agtggagtagtactccgctcacgttcgggtctgggaccaagctggagatcaaacgg

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AA

1D8 VL

divltqspitiaaspgekvtitcrassvsymywyqqksgasplwydtsklasgvpnrfsgsgtsyslaint  
metedaatyycqwsstpltfsggkcleikr

5

NT

1D8 scFv

aagcctatggattttcaagtcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggagtcgacatt  
10 gtgctcactcagcttcacaacacatagctgcattctcaggggagaaaggtcacatcacctgccgtgccagctcagtgtaagttacatgta  
ctgtgaccagcagaagtcaggcgccctccctaaactctgatttatgacacatccaagctggctctgtgagttccaaatgccttcagtgca  
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cacgttcgggtctgggaccagctggagatcaaacgggggtggcgtgctcggcggtgtggtgggtggcggtggcggtgacatcagggtg  
cagctgaaggaggcagtgacctggcctgtgtgcaaccgacacagacctgtccctcacatgactgtctctgggttctcatiaaaccagcgaatg  
15 gtgtacactggattcgaagccctcaggaagggtctgtggaatggaatgggaataatattatgatggagacagattataatcagcaatta  
aatccagactgagcatcagcagggaacacctcaagagccaagttttctanaaatcaacagctgtgcaaacgatgacacagccatgattac  
tgtgccagaatocactttgattactggggccaaggagtcagtggtcacagctctcctctgatca

AA

1D8 scFv

mdfqvfisfliliasvimrsgvdivltqspitiaaspgekvtitcrassvsymywyqqksgasplwydtskl  
asgvpnrfsgsgtsyslaintmetedaatyycqwsstpltfsggkcleikrgggsgsgsgsgsgsgsqvqlkagpglvqptqlslt  
ctvsgfsltsdgvhwrppgkglewmgiiydgtdynsaikrslsrdtsksqvfklinslqtdtdamyycaihfdywgqgvmm  
vtvss

25

NT

1D8 scFv IgG WTH WICH2CH3

aagcctatggattttcaagtcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggagtcgacatt  
30 gtgctcactcagcttcacaacacatagctgcattctcaggggagaaaggtcacatcacctgccgtgccagctcagtgtaagttacatgta  
ctgtgaccagcagaagtcaggcgccctccctaaactctgatttatgacacatccaagctggctctgtgagttccaaatgccttcagtgca  
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cacgttcgggtctgggaccagctggagatcaaacgggggtggcgtgctcggcggtgtggtgggtggcggtggcggtgacatcaggtg



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tgtgccgaatccatttattactggggccaaaggagtcattggtcacagtcctctgatctggagcccaaatctctgcacaaactcacaca  
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accctgaggtgacatgctgggtggtgacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcgtgaggtgca  
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aaccactacacgcagaagagcctctccctgtctcggggtaaagcggatcctcgaacctgctccatctggtggccattacctaatctcagta  
10 aatggaatttttgatagctgctcgtgacactactgcttgcgcccaagatgcagagagagaaggaggaatgagagattgagaagggaaggtg  
acgcctctgataaatcgata

AA

1D8 scFv IgG MTH MTCH2CH3-CD80

15 mdfqvqifslilisasvimrsgvdivltqspitiaaspgekvtitcrasssvsmywyqqksgasplkwiydtskl  
asgvpnrfsgsgtsyslainmetedaatyycqqwstpltfsgskleikrgggsgggsgggsggqqvqlkeagplvqptqlsl  
ctvsgfsltdgwhvhirppgkglewmgiiydggtynsaiksrslsrdtsksqvlkinsqltdtdamycarhfdywgqvm  
vtvssdlepkssdkthtspspapelggssvflfpkpkdltmistrpevtcvvvdshedpevkfnwyvdgvehnaktkpreeq  
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20 ngqpennyktppvlidsdgsfflyskltvdksrwqqgnvfscsvmhleahnhytqkslsispgkadpsnlflpswaitlsvngifvcc  
lycfaprcrrermerlresvrvp

NT

1D8 scFv IgG WTH WTCH2CH3-CD80

25 aagcttatggattttcaagtcagattttcagcttctgtaactcagtgcttcagtcagtaaatgctccagaggagtcgacatt  
gtgctcactcagctccacaacatactgctatccaggggaggaaggcaccatcacctgccgtgccagctccagtgtaagtacatgta  
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agcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtaacaaggtcacaagaagccctccagcccccacatcga  
gaaaacaa.tctccaaaggccaaggcgagcccgagaaccacaggtgtacaccctgcccccatccgggatgagctgaccaagaacca  
5 ggtcagcc tgcactgcctgtcaaaagcttctatccagcgacatcgccgtggaggtggagagcaatggggacccgggagaacaactaca  
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gtcttctcatgctccgtgatgatgaggtctgcacaaccactacagcagaagagcctctccctgctccgggtaaatgatctaga

AA

10 Anti human CD3 scFv WTH WTCH2CH3

Mdfqvqifsfllisasvimsrgvdiqmtqtsslsaslgdrvtircasqdirnylnwyqqkpdgtvkillytsrlh  
sgvpsrfsrgsgsgtdysltianlqpediatyfcqqnltlpwtfggggtklvtkrelggggsgggsgggsgsi devqlqsgpelvkpgas  
msckasgyrftgyivnwlkqshgknlewiglinpyklttynqkfkkgkatltvdksstaymellsdtsedavyyarsgyygsd  
wyfdwvgagttvtvssdqepksdktthtppcapellggpsvflfpkpkdltlmsrtpvtcvvvdshedpevkfnwyvdgv  
15 evhnahtktpreeqynstyrvvsvltvlhqdwingkeykckvsnkalpapiektiskakgqprepqvlytpprsdeltnqvsiltelvk  
gfypsdia vewesngqpennyktpvldsgsfllysklvtksrwqqgnvfscsvmhahlnhytqkslslspgk

NT

20 2H7-antiCD40 scFv MTH (SSS) MTCH2WTCH3

2h7-40.2.220Ig + restriction sites

aagcttgccgccatggattttcaagtcagattttcagcttctgtctaatacgtgttcacataatggcagaggaca  
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25 gcagtggtgtctgggaccttctactctctcaaatagcagngtggaggtgaagatgctgccacttattactccagcagtgaggattttaacc  
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15

AA

2H7-antiCD40 scFv MTH (SSS) MTCH2 WTCH3

2H7-40.2.220Ig

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20 lasgvparfsgsgstysltisrveadaatyycqawsfnptptgagtklelkgggsgggsgggssqaylqqsgaelvrpgasvk  
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30

NT

5B9 VH (includes the VH leader peptide)

atggctgtcttggggctgctcttctgctggtgacatttcaagctgtgtcctatccagggtgcagctgaagcagtcag  
gacctggcctatgtagctctccacagagcctgtccatcactgcacagtcctgtgttctcattaacactatgatgtgactgaggttcacca  
gtctccagcgaagggtctgagtgctggagtgatagtggtgggaatcacacagactaataatgcagcttctatccagactgacatca

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ataactacccttattacatgctctgactactgggtcagggaacctcagtcaccgtctctca

5B9 VH missing the leader:

5 cagggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagccctgctccacctgcacagctctg  
gtttctcatfaactaccatgctgtacactgggttcgccagctccaggaaagggtctggagtgctgggaagtataaggagtggtggaatca  
cagactataatgcagctttcatatocagactgagcatcaccaaggacgattccaagngccaagttttcttfaaaatgaacagctctgcaacctaa  
tgacacagccattattactgtgccagaaatgggggtgataactacccttattactatgctatgactactgggtcagggaacctcagtcacc  
gtctctca

10

AA

5B9 VH (includes leader peptide)

MAVLGLLFLVTFPSCVLSQVQLKQSGPGLVQSSQSL SITCTVSGFSLTTY  
AVHWVRQSPGKGLEWLGVIWWSGGITDYNAAFISRLSITKDDSKSQVFFKMNLSLPNDT  
15 AIYYCARNGGDNYPPYYAMDYWGQGTSTVTVSS

5B9 VH no leader peptide

QVQLKQSGPGLVQSSQSL SITCTVSGFSLTTYAVHWVRQSPGKGLEWLG  
VIWWSGGITDYNAAFISRLSITKDDSKSQVFFKMNLSLPNDTAIYYCARNGGDNYPPYYA  
20 MDYWGQGTSTVTVSS

NT

5B9 VL

atgagggttctctgcagcttctgggagctgctgtgctctgacccctgacccactgcagatattgtagacgcagg  
25 ctgcattctccaatccagtcactcttggaaacatcagcttccatctcctgcaggtctagtaagagcttccatagatattggcatcactatttga  
tttggtatctgcagaaaggccaggccagctcctcctcctctgatttaccagatgtccaaacctgcctcagagagtcocagacagggttcagtagcag  
tgggtcagggaactgatttcacactgaatcagcagagtgaggctgaggatgtgggtgtttattactgtgctcaaaatctagaactccgct  
cacgttctggctgctggaccagagctggagctgaaacgg

30

AA

5B9 VL

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5 NT  
5B9 scFv

AA  
5B9 scFv

20 MRFSAQLLGLLVLPVPGSTADIVMTQAAFSNPVTLGTASISCRSSKSLH  
SNGITYLYWYLQKPGQSPQLLIYQMSNLASGVDPDRFSSSGSGTDFTLRISRVEAEDGV  
YYCAQNLELPLTFGAGTKLELKRGGGSGGGGSGGGGSSQVQLKQSGPGLVQSSQSLSI  
TCTVSFGSLTTYAVHWVRQSPGKLEWLGVIIWSGGITDYNAAFISRLSITKDDSKSQVF  
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NT  
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15

AA

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20 YYCAQNLELPLTFGAGTKLELKRGGGSGGGSGGGSSQVQLKQSGPLVQSSQSLSI  
TCTVSGFSLTTYAVHWVRQSPGKLEWLGVIWSSGTTDYNAAFISRLSITKDDSKSQVF  
FKMNSLQPNDAIYYCARNGGDNYPIYYAMDYWGQGTSTVVSDDLPEPKSSDKTHTSP  
PSPAPELLGSSVFLFPPKPKDTLMISRTPPEVTCVVDVSHEDPEVKFNWYVDGVEVHN  
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAIEKTISKAKGQPR  
25 EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGS  
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30

NT

2e12 scFv WTH CH2 CH3 (2e12 scFv-WithIgG-CD80)

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20 2e12 scFv WTH CH2 CH3 2e12 scFv-WthIgG-CD80  
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NT  
30 2H7-human IgE Fc (CH2-CH3-CH4)  
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aattgttctctccagctccagcaatctgtctgcatctcaggggagaggtcacatgactgcaggccagctcaagtgaattgacat  
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atctaga

20 AA

2H7 scFv IgE (CH2-CH3-CH4)

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30 NT

2H7 scFv MH (SSS) MCH2WTCH3

aagcttgcccgcatggattttcaagtgacagattttcagcttctgctaatcagtgcttcagtcataattgccagaggaca  
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5 attaactacctatctgtacactgggttcgccagtcaccagaaagggtctggagtgctgggagtgatattggagtggtggaatcacagact  
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AA

15 **5B9 scFv MTHWTC2CH3**  
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 SNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDFRFSSSGSGTDFTLRISRVEAEDVGV  
 YYCAQNLELPLTFGATKLELKRGGGSGGGGSGGGSSQVQLKQSGPGLVQSSQSLSI  
 TCTVSGFSLTTYAVHWVRQSPGKLEWLGVIWSSGITDYNAAFISRLSITKDDSKSQVF  
 20 FKMNSLQPNDDTAIYYCARNGGDNYPIYYAMDYWGQGTSTVTVSSDQEPKSSDKTHTSP  
 PSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN  
 AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTKISKAKGQPR  
 EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGS  
 FFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK

25

Human IgG1 hinge mutations

30 **2H7 scFv- MTH (CSS) WTCH2CH3**

Nucleotide:

aagcttgccgccatgatttcaagtcagatttctcactgctaaicagtgcttcagtcataattgccagaggaca  
 aatgtgtctctccagctctccagcaaatctgtctgcatctccagggagagaaggtcacatgacttgcagggcagctcaggtgtaagttaacat

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10 caa.ctggtac.gtg.gacggc.gtg.gagtg.cataatg.ccaag.gacaa.gccgc.gggga.ggagcag.tacaac.agc.acgtacc.gtg.tgctcagc  
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2H7 scFv- MTH (CSS) WTCH2CH3 protein:

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25 2H7 scFv- MTH (SCS) WTCH2CH3:

Nucleotide:

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2H7 scFv- MTH (SCS) WTCH2CH3 Protein:

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2H7 scFv- MTH (SSC) WTCH2CH3:

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HIgGMHcys2

gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tct oca ccg tgc

HIgGMHcys3

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2H7 scFv MTH (SSS) WTCH2MTH3A405

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AA

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a s r q e p s g t t t f a v i s l i r v a e d w k k g d t f s c m v g h e a l p l a f l q k t i d r l a g k p t h v n v s v m a e v d a d p s n n l p s w a i l i s v n  
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acctgccagggcctctcatgggtccacgaccaagaccagcggcccgcgtgctgccccggaagcttatgctgtttgcgacccggagtg  
10 gccggggagccgggacaagcgcacctcgcctgctgacccaacttcatgctgaggaacatctcgtgtgagtggtgacacacgag  
gtgagctcccggaagcggcggcacagcacgacgacgccccgaagaccagggtctcctctctcgtcttcacccgcctggaggtga  
ccaaggccgaatgggagcagaagaatgagttcatctgcccgtgcagtcctagggcagcgagcccccacagaccgtccagcagcgggt  
gtctgtaatacccgtaaaagggaatcctcgaa

15

AA

human IgE Fc (CH2-CH3-CH4) ORF:

dhvcsrdtpptvkilqssedggghfpptqlclvsytpgtinitwledgqvmdvdlstastqegelastqseltl  
sqkhlwlsdrtycqvttyqhtfedstkkcadnsnprgvsaylsrpspdlfrksptitclvddlpskgtnlvtwsragkpnvhnstrkee  
20 kqrngltlvtstlpvgtrdwiagetqqrviplphlpralmrstktsgrpraapevyafatpewpgsrdrktlaclqnmfpedisvqwh  
nevqlpdarhsttpkrktgsgffvfrlvttraewqkdeficravheaaspsqtvqrvsvnpgkadps

25

NT

1D8 scFv-human IgE Fc (CH2-CH3-CH4)-CD80

aagcttatggattttcaagtgcagattttcagctctctctaatactgcttcaagtcataatgtccagaggagtcgacatt  
gtgctcactcagctccacaacactagctgcatlccaggggagaaggtcacatcacctgccgtgccagctccagtgtaagttaactgta  
ctgtaccagcagaagtcaggcgccctccctaaactctgatttatgacacatccaagctggcttctggagttccaatlcgctcagtgcca  
20 tgggtctgggacatcttattctctcgaatcaacacatggagactgaagatgctgccattattactctcagcagctggagtagtactccgct  
cagctcgggtgtcgggaccaagctggagatcaaacgggggtggcggtggctcggggcggtggtggcggcggtgacatcaggtg  
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15

mdfqvqj fsfliliasvinsrgvdivlqspitiaaspgelkvtilcrassvsvmywyvqqksasgplwiydtskl  
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edstckkacdsnpgrsvaisrpspdlfirsptitclvdlapskgtnlwtvsragkpvnhstrkeekqmgltvt  
tyqcrvthphlpralmrsttktsppraepvyafatpewpgsrkrlaclqinfmpedisvqwlhnevqlpar  
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nv

25

## 5B9-IgA-H IgA-T4-CD80

30

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 5 tctgatagccaggtccctcaactccactacccatctccctcaactccactacccatctccctcatgctgccacccccgactgtcactgc  
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 attttgtgatagctgctgcagcactactgctttgccccaagatgcagagagagaaagggaagaaagagatgagaagaaaggtgtacgcc  
 15 ctgtataaatcgatgc

AA

5B9-IgAH IgA-T4-CD80

mrfsaqlllglvlwipgstadivmiquaafsnpvtlgtasiscsrsskllhsngitylywylqkpgqspqliiyqms  
 20 nlasgvprdfsssgsgtdflrisrveadvgvyvcaqnlelplltfgagtklelkrggsgggsgggsgggssqvlkqsgplvqssql  
 sitctvsgfsltyavhwrpyspgkglewlgviwsggtdynaafisrlsitkddsksqvfkmmnslqpdntaiyyccarnngdnyppy  
 amdywgggtsvtvssdqpvpstppstppstppscchprlsihrapledlllgseailctgltdrdsagvftvtpssgksavagpp  
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 sqelprekyltwasrqepsqgtttfavtsilrvaedwkgdgtfscmvghaelplafitqktidrlagkphvnvsvmaevdadpsnnl  
 25 lpswaitlisvngifviclctyefaprcrrrrrrrrrrresvrv

NT

5B9-scFv-human IgE Fc (CH2-CH3-CH4)-CD80

aagcttgcgcgccatgaggttctctgctcagcttctggggctgctgtcgtcttgaccctcgatccactgcagatattgt  
 30 gatgacgcagcgtcattctccaatccagctactcttgaacatcagcttccatctcctgcaggtctagtaagaagctcctcatagatgaatgac  
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 gttcagtagcagctgtgtcagaagcatttcacactgagaaatcagcagagtgaggctgagagtgatgggtttattactgtgtcctaaatct

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20

5B9-scFv-human IgE Fc (CH2-CH3-CH4)-CD80

2e12-scFv-IgAH IgA-T4-CD80

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aagcttatggatttcaagtcagattttcagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatt

25

2e12-scFv-human IgE Fc (CH2-CH3-CH4)-CD80

30



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isvqwlhnevqlpdarhstprkrtksggffvfrlevtraewqkdeficravheaaspsqtvqravsvnpgkadpsklpswaitlsv  
ngifvicltyclaprcrermerlrrsvrpv

NT

5 500A2 scFv

atgttgatatactcagctcctgggcttttactcttcttgattcagcctccagaagtgacatagtgtgactcagactc  
cagccactctgtctctaattcctggagaaagagtcacaatgacctgtaagaccagtcagaatattggcacaaatcttactcggatcaccaaa  
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15 AA

500A2 scFv

mlytsqllgllfwisarsdivltqtpatlslipegervtmctksqniqtilhwyhqkpkpeapralikyassqisipip  
srfsgsgsetdfinnlepdidgiyycqqsrsrwpvtfgptkcleikrsgggsgggsgggsgvklqsgselgkpgasvklsccts  
20 gyiftdhyiswvkqkpgeslqwignvvyggnggtsynqkfgkattlvdkisstaymelsltsedsaiyyccarppvatghamdywg  
qgiqvtvssd

5' oligo:

25 Name : hIgAbel5  
Sequence : GTTGTGTGATCAGCCAGTTCCTCAACTCCACCTACC

3' oligo:

Name : IgA3BB  
30 GTTGTGTTTCGAAGGATCCCGCTCCACCTCCGCCATGACAACAGA

5' oligo:

Name : IgGWT3

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GT'TGTTTTCGAAGGATCCGCTTTACCCGGAGACAGGGAGAGGCTCTT

3' oligo:

Name : hIgGWT5

5

GTTGTTAGATCTGGAGCCCAAATCTTGTGACAAAACACACATG

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5' oligo:

Name : FADD5

Sequence

5 GTTGTGGATCCTTCGAACCCGTTCTGGTGTCTGCACTCGGTGTCTG

3' oligo:

Name : FADD3

Sequence

10 GTTGTATCGATCTCGA GTTATCAGGACGCTTCGGAGGTAGATGCGTC

FADD-CSSCFV:

Gtggatctctgaacccgttctctggtgctgctgactcgggtgtcgtccagcctgtcagcagcgagctgaccgagct  
caagttctatgctctggcgctggtggcaagcgcaagctggaagcgtgagagcggcctagacctcttctcatgctgctggagcaga  
15 acgacctggagccccggcgacaccgagctcctgctgagcagctgctcgtccctgctggcgccacgacctgctgctggcgctgacgactt  
cgagcgccccggcgctggcgccggcgctgctggcggaagacctgtgtgacgatttaacgtcatatgtgataatgtgggaaagat  
tggagaaggctgctgctgctcagctcaaatgtctcagacaccagatcgacagcagcagacagataccccgcaacctgacagagcgtgt  
gcgggagtcactgagaatctggaagaacacagagaaggagaacgcacagtgccacactggtggggctctcaggtcctgccagatg  
aacctgtgtgctgacctggtacaagagggtcagcagccgtgacctcagaacaggagtggggccatgtccccgatgctatggaactc  
20 agacgcatctacctcgaagcgtcctgataactcgagatcgatacaaac

Peptide sequence:

vdpsnpflvllhsvssslssstcltkfclgrvgrkrklervqsgldlfsmlleqndlepghltellrelaslrhdllrrv  
ddfeagaaagaapgeedlcaafnvcnvgkdwrrlarqlkvsdtkidsiedryprnltervreslrwknktenatvahlgalscq  
25 mnlvadlvqevqqardlqnrgamspmswnsdastseas

Name : HCD28tm5B

GTTGTGGATCCTCCCTTTTGGGTGTCTGGTGGTGGTTGGTGTCTCTGGCCT

30 GCTATAGCTTG

Name : HCD28tm3S

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GTTGTTTCGAACCCAGAAAATAATAAAGGCCACTGTTACTAGCAAGCT  
ATAGCAAGCCAG

HCD28tm5'  
5 GTTGTGGATCCTCCCTTTTGGGTGCTGGTGGT  
HCD28tm3'  
GTTGTTTCGAACCCAGAAAATAATAAAGGCCAC

10 HCD80tm5'  
GTTGTGGATCCTCCTGCTCCCATCCTGG  
HCD80tm3'  
GTTGTTTCGAACGGCAAAGCAGTAGGTCAGGC

15 Name : MFADD5BB  
Sequence :  
GTTGTGGAATCCTTCGAACCCATTCTGTGTGCTGCTGCAC'TCGCTG

20 Name : MFADD3XC  
Sequence :  
GTTGTTATCGATCTCGAGTCAGGGTGT'TTCTGAGGAAGACAC

25 Murine FADD Nucleotide sequence (full length, but without flanking -Ig or  
transmembrane sequences):

Gtggatccttcgaacatggaccattcctggtgctgctgcactcgtgtccggcagcctgtcgggcaacgatcgtat  
ggagctcaagtctctgtgccgcgagcgcgtgagcaaacgaaagctggagcgcgtgcagagtggtccctggacctgttcacgggtgctgctg  
agcagaacgacctggagcgcggggcacaccgggctgctgcgcgagttgctggcctcgtgcgcgcgacacgatctactgcagcgcctgga  
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30 agagactggaaaagactgcccgcgagctgaaggtgtctgaggccaagatggatggattgaggagaagtacccccgaagtctgagtg  
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Murine FADD

vdpsnmdpfllvllhsisgslsgndlmelkflcrervskrklerlvqsgldlftvlleqndlerghtglirellaslrhddll  
qrlldfeagtataappgeadlqvafdivcdnvgrdwkrlelkvseakmdgieekyprslservreslkvwknackknasvaglvk  
5 alrtcrlnlvadlvecagesvsksenmspylrdstvsstetp

Name : MCASP3-5

10 Sequence :

GTTGTGGATCCTTCGAACATGGAGAACAACAAAACCTCAGTGGATTCA

Name : MCASP3-3

Sequence :

GTTGTTATCGATCTCGAGCTAGTGATAAAAGTACAGTTCTTTCGT

15

Name : mcasp8-5

Sequence :

20 GTTGTTTCGAACATGGATTTCAGAGTTGTCTTTATGCTATTGCTG

Name : mcasp8-3

Sequence :

GTTGTTATCGATCTCGAGTCATTAGGGAGGGAAGAAGAGCTTCTTCCG

25

Name : hcasp3-5

Sequence :

GTTGTGGATCCTTCGAACATGGAGAACAACGAAACTCAGTGGAT

Name : hcasp3-3

30 Sequence :

GTTGTTATCGATCTCGAGTTAGTGATAAAATAGAGTTCTTTTGTGAG

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PCT/US2003/024918

Name : hcaspr8-5

Sequence :

GTTTGTGGATCCTTCGAACATGGACTTCAGCAGAAATCTTTATGAT

Name : hcaspr8-3

5

Sequence :

GTTTGTATCGATGCATGCTCAATCAGAAGGGAAGACAAGTTTTTTCT

HuIgGMHWC

10

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tcc cea gen cct gaa  
ctc ctt ggt g ga ccg tca gtc ttc c

NT

15

2H7-human IgE (CH2-CH3-CH4)

aagcttgccgccatggattttcaagtgacagatttcagcttctgtaatacagtgcttcagtcataattgccagaggaca  
aatgttctctccacgtctccagcaatctgtctgcatctccaggggagaaggtcacaatgacttgacggccagctcaagtgtaattacat  
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20 caccoccttctgctgctgggaccacgc tggagctgaaggtggcggtgctggcggtgctggatctggaggagtggtggagctcta  
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cacagcacgacgacgccccgaagaccaaggctccgctctctcgtcttcagccgctggagggtaccaggggccgaantgggagcaga  
aagatgagttcatctgccgtgcatgcatgagcagcgagccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaatgata  
atctaga

5

AA

2H7 scFv IgE (CH2-CH3-CH4)

mdfqvqifsfllisaviiargqivlsqspailsaspgekvtmtrassvsmhwyqqkpgsspkpwiypasn  
lasgvparfsgsgsgtsylstrveadaatyycqqwsmfpptfagtklelkgggsgggsgggssqaylqqsgaelvrpgasvk  
msckasgytfsynmhvkvtpqrqglewigaiypngdtsynqkfkkgkatltvdkssstaymqlsslsedsavfyfcarvvyysns  
10 ywyfdvwtgcttvtvshvcsrdlfpptvkilqsscdggghfpptqllclvsgytpgtinitwiedgqvmvdvlstasttqgelastqs  
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wlhnevqlpdrhsttpqrktksggffvfrlevtraewekdeficra vheaaspsqlqvrasvnpkg

15

NT

2H7 scFv MH (SSS) MCH2.WTCH3

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20 aattgttctctccagctctccagcaatcctgtctgcatctccagggaagaaggtcaaatgacttgcaggcgcagctcaagtgtaaagtacat  
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25 acaatgatcactgggtaaagcagacacctagacaggccctggaatggattggaagctattttacaggaaatggtgatacttctcacaatcag  
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AA

5 2H7 seFv MH (SSS) MCH2WTCH3

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msckasgytfsynmhwwkvtprqlgwigaiypngdtsynqkfkgkatitvdkssstaymqllsslsedsavyfcarvvyysns  
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10 evhnaktkpreeqynstnyrvsvtlvhqdwlngkeykckvsnkalpapiektiskakgqprepqvyltppsrldtknqslctlvk  
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NT

15 5B9 seFv MTHWTCH2CH3

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gttcagtagcagtggtgcaggaaactgattcacactgagaatcagcagagtgaggctgaggatgtgggtgtttattactgtgtcctaaatct  
agaacttcgcctcagctgttctgctgggaccagctggagctgaaacgggggtggcggctggcggcggcggcggcggcggcggcggcggc  
20 ggatcgtcacaggtgcagctgaagcagtcaggacctggcctagtgagctcctcacagagcctgtcctacacagctgcacagctctctgtttctc  
attaactcacctatgctgtacactgggttcgccagctccaggaaaggctgtggagtgctgggagtgatgagtggtggaatcacagact  
ataatgcagcttcatatccagactgagcatcaccaaaggagttccaagagccaaagtttctttaaatgaacagctctgcacactaatgacac  
agccatttattactgtgccagaatgggggtgataactaccccttattactatgctatggactactgggctcaggaaacctcagtcaccgtctcc  
tctgatacaggagcccaaatcttctgacaaaactcacacatcccacagctcccagcacctgaaactcctgggggagccgtgacttctctctt  
25 ccccccacaaaccaaagcagaccctcatgatctccggacccttgaggctcacatgcgtgtgtgtgagcgtgagccacgaagaccctgag  
gtc-aagtccaactggtactggagcggcgtggaggtgcataatgccaaagacaagccgcggggagagcagtaacaacagcagtaaccgtg  
tggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtaacagtgcaagctctccaaacaaagccctccagccccc  
atcgagaaaaaactcctcaagcccaaaaggcagccccgagaaccacaggtgtacacccctgccccctccgggatgagctgacacaa  
accaggtcagcctgacgtgctgtgtaaaaggcttctatccacgcagacatccgctggagtgaggagcaalgggcagccggagaacaa  
30 ctaacagaccagcctcccgctgctgactccagggctccttctctctacagcaagctcaccgtggcacaagagcagcgtggcagcagg  
gaacgtctctcatgctcctgtagtcatgaggtctgcacaaacactacacgcagaagagccttccctctgctccgggtaaatgatctaga

AA



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5 mrfsaqllglvlwipgstadivmtqaafsnvptlgtasasircsskllshngitylywylqkpgsqpalliyqms  
nlasgvpdfrsssgtdfltrisveacdgvvycaqnlelplftgagtklelkrggsgsgsgsgsgsgsqvqlkqsgplvssqs  
sitcvtsgfslityavhwvrqspgkglewlgiwvsggittynaafsrslitkddksqvfkknnslqpnidaitycarnegdnypyy  
amdywgqgtsvtvssdgpeksdkthtspspapellggpsvflppkpkdtlmsirpvtvcvvdvshdedpevkfnwvydgv  
vhnaktprceeqnnyrvsvslthpdlwngkeyckvskvsnkalpaktiskagqprepqvvtlppsrldtqnvsllcivkgf  
ypsdiavewsnqgnsterrypvtdlpsdsgfsskylvtdrswagvncfscvmeahlnhvtqslisslspek

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the present invention is not limited except as by the appended claims.

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# CLAIMS

1. A binding domain-immunoglobulin fusion protein, comprising:
  - 5 (a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide is selected from the group consisting of (i) a wild-type human IgG1 immunoglobulin hinge region polypeptide, (ii) a mutated human IgG1 immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human  
10 IgG1 immunoglobulin hinge region polypeptide contains two cysteine residues and wherein a first cysteine of the wild-type hinge region is not mutated, (iii) a mutated human IgG1 immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human IgG1 immunoglobulin hinge region polypeptide contains no more than one cysteine residue, and  
15 (iv) a mutated human IgG1 immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human IgG1 immunoglobulin hinge region polypeptide contains no cysteine residues;
    - (b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to  
20 the hinge region polypeptide; and
    - (c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide,  
wherein:
      - (1) the binding domain-immunoglobulin fusion protein is capable of at least  
25 one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation, and
      - (2) the binding domain polypeptide is capable of specifically binding to an antigen.
- 30 2. An isolated binding domain-immunoglobulin fusion protein comprising: a) a binding domain polypeptide capable of specifically binding to an antigen; b) an immunoglobulin hinge region polypeptide fused to said binding domain polypeptide, said immunoglobulin hinge region polypeptide comprising first, second, and third cysteine residues, where said first cysteine residue

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is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said first cysteine residue is not mutated and one or both of said second and third cysteine residues is substituted or deleted; and c) an N-terminally truncated immunoglobulin heavy chain constant region polypeptide fused to said immunoglobulin hinge region polypeptide, wherein the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation.

3. The binding domain-immunoglobulin fusion protein of claim 1 or 2 wherein the binding domain polypeptide comprises at least one immunoglobulin variable region polypeptide that is selected from the group consisting of an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

4. A binding domain-immunoglobulin fusion protein according to claim 3 which comprises an immunoglobulin heavy chain variable region polypeptide, wherein said heavy chain variable region polypeptide is a human immunoglobulin heavy chain variable region polypeptide comprising a mutation at an amino acid at a location corresponding to amino acid position 11 in the first framework region of the heavy chain variable region.

5. A binding domain-immunoglobulin fusion protein according to claim 3 which comprises a polypeptide having a sequence selected from the group consisting of SEQ ID NO:\_\_\_ and SEQ ID NO:\_\_\_.

6. The binding domain-immunoglobulin fusion protein of claim 3 wherein the immunoglobulin variable region polypeptide is derived from a human immunoglobulin.

7. The binding domain-immunoglobulin fusion protein of claim 3 wherein the immunoglobulin variable region polypeptide comprises a humanized immunoglobulin polypeptide sequence.

8. The binding domain-immunoglobulin fusion protein of claim 3 wherein the immunoglobulin variable region polypeptide is derived from a murine immunoglobulin.

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9. The binding domain-immunoglobulin fusion protein of claim 1 or 2 wherein the binding domain polypeptide comprises:
  - (a) at least one immunoglobulin light chain variable region polypeptide;
  - (b) at least one immunoglobulin heavy chain variable region polypeptide; and
  - 5 (c) at least one linker polypeptide that is fused to the polypeptide of (a) and to the polypeptide of (b).
10. The binding domain-immunoglobulin fusion protein of claim 9 wherein the immunoglobulin light chain variable region and heavy chain variable region polypeptides are derived from human immunoglobulins.
11. The binding domain-immunoglobulin fusion protein of claim 9 wherein the linker polypeptide comprises at least one polypeptide having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO: \_\_].
12. The binding domain-immunoglobulin fusion protein of claim 9 wherein the linker polypeptide comprises at least three repeats of a polypeptide having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO: \_\_].
- 20 13. A binding domain-immunoglobulin fusion protein according to claim 9 wherein the linker comprises a glycosylation site.
14. The fusion protein of claim 13 wherein the glycosylation site is selected from the group consisting of an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site and a phosphoglycation site.
- 25 15. The binding domain-immunoglobulin fusion protein of claim 1 wherein at least one of the immunoglobulin heavy chain CH2 constant region polypeptide and the immunoglobulin heavy chain CH3 constant region polypeptide is derived from a human immunoglobulin heavy chain.
- 30

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16. The binding domain-immunoglobulin fusion protein of claim 1 wherein the immunoglobulin heavy chain constant region CH2 and CH3 polypeptides are of an isotype selected from the group consisting of human IgG and human IgA.
17. The binding domain-immunoglobulin fusion protein of claim 1, 2 or 73 wherein the antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, L6, CD2, CD28, CD30, CD40, CD50 (ICAM3), CD54 (ICAM1), CD80, CD86, B7-H1, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, CD19, CD3, CD4, CD25, CD8, CD11b, CD14, CD25, CD56 and CD69.
18. The binding domain-immunoglobulin fusion protein of claim 1 or 2 wherein the binding domain polypeptide comprises a CD154 extracellular domain.
19. The binding domain-immunoglobulin fusion protein of claim 1 or 2 wherein the binding domain polypeptide comprises a CD154 extracellular domain and at least one immunoglobulin variable region polypeptide.
20. The binding domain-immunoglobulin fusion protein of claim 1 or 2 wherein the binding domain polypeptide comprises a CTLA-4 extracellular domain.
21. The binding domain-immunoglobulin fusion protein of claim 2 or 20 wherein at least one of the immunoglobulin heavy chain constant region polypeptides selected from the group consisting of a CH2 constant region polypeptide and a CH3 constant region polypeptide is a human IgG1 constant region polypeptide.
22. The binding domain-immunoglobulin fusion protein of claim 20 wherein at least one of the immunoglobulin heavy chain constant region polypeptides selected from the group consisting of a CH2 constant region polypeptide and a CH3 constant region polypeptide is a human IgA constant region polypeptide.
23. A binding domain-immunoglobulin fusion protein, comprising:
  - (a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide;

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(b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide; and

(c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide,

5 wherein:

(1) the binding domain polypeptide comprises a CTLA-4 extracellular domain that is capable of specifically binding to at least one CTLA-4 ligand selected from the group consisting of CD80 and CD86,

(2) the immunoglobulin hinge region polypeptide comprises a polypeptide  
10 that is selected from the group consisting of a human IgA hinge region polypeptide and a human IgG1 hinge region polypeptide,

(3) the immunoglobulin heavy chain CH2 constant region polypeptide comprises a polypeptide that is selected from the group consisting of a human IgA heavy chain CH2 constant region polypeptide and a human IgG1 heavy chain CH2 constant region  
15 polypeptide,

(4) the immunoglobulin heavy chain CH3 constant region polypeptide comprises a polypeptide that is selected from the group consisting of a human IgA heavy chain CH3 constant region polypeptide and a human IgG1 heavy chain CH3 constant region  
polypeptide, and

(5) the binding domain-immunoglobulin fusion protein is capable of at least  
20 one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation.

24. A binding domain-immunoglobulin fusion protein, comprising:

(a) a binding domain polypeptide that is fused to an immunoglobulin hinge region  
25 polypeptide, wherein said hinge region polypeptide comprises a human IgE hinge region polypeptide;

(b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a human  
30 IgE CH2 constant region polypeptide; and

(c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a human IgE CH3 constant region polypeptide

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wherein:

- (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and induction of an allergic response mechanism, and
  - 5 (2) the binding domain polypeptide is capable of specifically binding to an antigen.
25. A binding domain-immunoglobulin fusion protein according to claim 24 that comprises a human IgE CH4 constant region polypeptide.
- 10 26. The binding domain-immunoglobulin fusion protein of claim 24 wherein the antigen is a tumor antigen.
27. A binding domain-immunoglobulin fusion protein, comprising:
- 15 (a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of specifically binding to at least one antigen that is present on an immune effector cell and wherein the hinge region polypeptide comprises a polypeptide selected from the group consisting of a human IgA hinge region polypeptide, a human IgG hinge region polypeptide, and a human IgE hinge region
- 20 polypeptide;
- (b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH2 constant region polypeptide, a human IgG CH2 constant region polypeptide, and a human IgE CH2 constant
- 25 region polypeptide;
- (c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH3 constant region polypeptide, a human IgG CH3 constant region polypeptide, and a human IgE CH3 constant
- 30 region polypeptide; and
- (d) a plasma membrane anchor domain polypeptide.

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28. The binding domain-immunoglobulin fusion protein of claim 27 wherein the membrane anchor domain polypeptide comprises a transmembrane domain polypeptide.
29. The binding domain-immunoglobulin fusion protein of claim 27 wherein the membrane anchor domain polypeptide comprises a transmembrane domain polypeptide and a cytoplasmic tail polypeptide.
30. The binding domain-immunoglobulin fusion protein of claim 29 wherein the cytoplasmic tail polypeptide comprises an apoptosis signaling polypeptide sequence.
31. The binding domain-immunoglobulin fusion protein of claim 30 wherein the apoptosis signaling polypeptide sequence is derived from a receptor death domain polypeptide.
32. The binding domain-immunoglobulin fusion protein of claim 31 wherein the death domain polypeptide comprises a polypeptide selected from the group consisting of an ITIM domain, an ITAM domain, FADD, TRADD, RAIDD, CD95 (FAS/ Apo-1), TNFR1 and DR5.
33. The binding domain-immunoglobulin fusion protein of claim 30 wherein the apoptosis signaling polypeptide sequence comprises a polypeptide sequence derived from a caspase polypeptide that is selected from the group consisting of caspase-3 and caspase-8.
34. The binding domain-immunoglobulin fusion protein of claim 27 wherein the plasma membrane anchor domain polypeptide comprises a glycosyl-phosphatidylinositol-linkage polypeptide sequence.
35. The binding domain-immunoglobulin fusion protein of claim 27 wherein the antigen that is present on an immune effector cell is selected from the group consisting of CD2, CD28, CD30, CD40, CD50 (ICAM3), CD54 (ICAM1), CD80, CD86, B7-H1, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, CD19, CD20, CD22, CD37, L6, CD3, CD4, CD25, CD8, CD11b, CD14, CD25, CD56 and CD69.
36. The binding domain-immunoglobulin fusion protein of claim 27 wherein the human IgG is human IgG1.



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37. A binding domain-immunoglobulin fusion protein, comprising:

(a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of specifically binding to at least one antigen that is present on a cancer cell surface and wherein the hinge region polypeptide comprises a polypeptide selected from the group consisting of a human IgA hinge region polypeptide, a human IgG hinge region polypeptide, and a human IgE hinge region polypeptide;

(b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH2 constant region polypeptide, a human IgG CH2 constant region polypeptide, and a human IgE CH2 constant region polypeptide;

(c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH3 constant region polypeptide, a human IgG CH3 constant region polypeptide, and a human IgE CH3 constant region polypeptide; and

(d) a plasma membrane anchor domain polypeptide.

38. The binding domain-immunoglobulin fusion protein of claim 37 wherein the membrane anchor domain polypeptide comprises a transmembrane domain polypeptide.

39. The binding domain-immunoglobulin fusion protein of claim 37 wherein the membrane anchor domain polypeptide comprises a transmembrane domain polypeptide and a cytoplasmic tail polypeptide.

40. The binding domain-immunoglobulin fusion protein of claim 37 wherein the membrane anchor domain polypeptide comprises a glycosyl-phosphatidylinositol-linkage polypeptide sequence.

41. The binding domain-immunoglobulin fusion protein of claim 37 wherein the human IgG is human IgG1.

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42. A binding domain-immunoglobulin fusion protein, comprising:

(a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide comprises a wild-type human IgA hinge region polypeptide;

5 (b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a human IgA CH2 constant region polypeptide; and

(c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a  
10 polypeptide selected from the group consisting of (i) a wild-type human IgA CH3 constant region polypeptide and (ii) a mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain,

wherein:

(1) the binding domain-immunoglobulin fusion protein is capable of at least  
15 one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation, and

(2) the binding domain polypeptide is capable of specifically binding to an antigen.

20 43. The binding domain-immunoglobulin fusion protein of claim 42 wherein the mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain is selected from the group consisting of (i) a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:\_\_\_ and (ii) a polypeptide comprising an amino acid sequence as set forth in  
SEQ ID NO:\_\_\_.

25

44. A binding domain-immunoglobulin fusion protein, comprising:

(a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide;

(b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to  
30 the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a llama CH2 constant region polypeptide that is selected from the group consisting of a llama IgG1 CH2 constant region polypeptide, a llama IgG2 CH2 constant region polypeptide and a llama IgG3 CH2 constant region polypeptide; and

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- (c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a llama CH3 constant region polypeptide that is selected from the group consisting of a llama IgG1 CH3 constant region polypeptide, a llama IgG2 CH3 constant region polypeptide and a llama IgG3 CH3 constant region polypeptide

wherein:

- (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and induction fixation of complement, and
- (2) the binding domain polypeptide is capable of specifically binding to an antigen.

45. A binding domain-immunoglobulin fusion protein according to claim 44 wherein the immunoglobulin hinge region polypeptide, the llama CH2 constant region polypeptide and the llama CH3 constant region polypeptide comprise sequences derived from a llama IgG1 polypeptide and wherein the fusion protein does not include a llama IgG1 CH1 domain.

46. A binding domain-immunoglobulin fusion protein according to any one of claims 1, 2, 23, 24, 27, 37, 42 or 44 wherein the hinge region polypeptide is mutated to contain a glycosylation site.

47. The fusion protein of claim 46 wherein the glycosylation site is selected from the group consisting of an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site and a phosphoglycation site.

48. A binding domain-immunoglobulin fusion protein according to any one of claims 1, 2, 23, 24, 27, 37, 42 or 44 wherein the binding domain polypeptide comprises two or more binding domain polypeptide sequences wherein each of said binding domain polypeptide sequences is capable of specifically binding to an antigen.

49. A binding domain-immunoglobulin fusion protein, comprising:

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- (a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein the hinge region polypeptide comprises an alternative hinge region polypeptide sequence;
- (b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide; and
- (c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide,
- wherein:
- (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation, and
- (2) the binding domain polypeptide is capable of specifically binding to an antigen.
50. A binding domain-immunoglobulin fusion protein, comprising:
- (a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of specifically binding to at least one antigen that is present on a cancer cell surface and wherein the hinge region polypeptide comprises an alternative hinge region polypeptide sequence;
- (b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH2 constant region polypeptide, a human IgG CH2 constant region polypeptide, and a human IgE CH2 constant region polypeptide;
- (c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH3 constant region polypeptide, a human IgG CH3 constant region polypeptide, and a human IgE CH3 constant region polypeptide; and
- (d) a plasma membrane anchor domain polypeptide.

51. A binding domain-immunoglobulin fusion protein according to either claim 49 or claim 50 wherein the alternative hinge region polypeptide sequence comprises a polypeptide sequence

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of at least ten continuous amino acids that are present in a sequence selected from the group consisting of SEQ ID NOS: \_\_-\_\_.

52. An isolated polynucleotide encoding a binding domain-immunoglobulin fusion protein  
5 according to any one of claims 1, 2, 23, 24, 27, 37, 42, 44, 49 and 50.

53. A recombinant expression construct comprising a polynucleotide according to claim 52 that is operably linked to a promoter.

10 54. A host cell transformed or transfected with a recombinant expression construct according to claim 53.

55. A method of producing a binding domain-immunoglobulin fusion protein, comprising the steps of:

(a) culturing a host cell according to claim 54 under conditions that permit  
15 expression of the binding domain-immunoglobulin fusion protein; and

(b) isolating the binding domain-immunoglobulin fusion protein from the host cell culture.

56. A pharmaceutical composition comprising a binding domain-immunoglobulin fusion  
20 protein according to any one of claims 1, 2, 23, 24, 42, 44, 49 or 50 in combination with a physiologically acceptable carrier.

57. A pharmaceutical composition comprising an isolated polynucleotide encoding a binding  
25 domain-immunoglobulin fusion protein according to any one of claims 1, 2, 23, 24, 27, 37, 42, 44, 49 and 50 in combination with a physiologically acceptable carrier.

58. The use of a compound according to claim 1 in the preparation of a medicament for the  
treatment of a subject having or suspected of having a malignant condition or a B-cell disorder,  
comprising administering to a patient a therapeutically effective amount of a pharmaceutical  
30 composition selected from the group consisting of the pharmaceutical composition of claim 56 and the pharmaceutical composition of claim 57.

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59. The use of claim 58 wherein the malignant condition or B-cell disorder is selected from the group consisting of a B-cell lymphoma and a disease characterized by autoantibody production.
- 5 60. The use of claim 58 wherein the malignant condition or B-cell disorder is selected from the group consisting of rheumatoid arthritis, myasthenia gravis, Grave's disease, type I diabetes mellitus, multiple sclerosis and an autoimmune disease.
61. The use of claim 58 wherein the malignant condition is selected from the group  
10 consisting of melanoma, carcinoma and sarcoma.
62. A binding domain-immunoglobulin fusion protein according to claim 2 wherein said second cysteine residue is substituted or deleted and said third cysteine residue is not substituted or deleted.
- 15 63. A binding domain-immunoglobulin fusion protein according to claim 62 wherein said second cysteine residue is substituted and not deleted.
64. A binding domain-immunoglobulin fusion protein according to claim 63 wherein said  
20 second cysteine is replaced with serine.
65. A binding domain-immunoglobulin fusion protein according to claim 2 wherein said third cysteine residue is substituted or deleted and said second cysteine residue is not substituted or deleted.
- 25 66. A binding domain-immunoglobulin fusion protein according to claim 65 wherein said third cysteine residue is substituted and not deleted.
67. A binding domain-immunoglobulin fusion protein according to claim 66 wherein said  
30 third cysteine is replaced with serine.
68. A binding domain-immunoglobulin fusion protein according to claim 2 wherein said second cysteine and said third cysteine residues are both substituted or deleted.

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69. A binding domain-immunoglobulin fusion protein according to claim 68 wherein said second cysteine and said third cysteine are substituted.
- 5 70. A binding domain-immunoglobulin fusion protein according to claim 68 wherein said second cysteine and said third cysteine are replaced with serine.
71. A binding domain-immunoglobulin fusion protein according to any one of claims 2, 62, 63, 64, 65, 66, 67, 68, 69, or 70 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.
- 10 72. A binding domain-immunoglobulin fusion protein according to any one of claims 2, 62, 63, 64, 65, 66, 67, 68, 69, or 70 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.
- 15 73. An isolated binding domain-immunoglobulin fusion protein comprising: a) a binding domain polypeptide capable of specifically binding to an antigen, said binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide, wherein the leucine at position 11 in the first framework region of said heavy chain variable region polypeptide is substituted or deleted; b) an immunoglobulin hinge region polypeptide fused to said binding domain polypeptide; and c) an N-terminally truncated immunoglobulin heavy chain constant region polypeptide fused to said immunoglobulin hinge region polypeptide, wherein the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation.
- 20
- 25

30

# FIG. 1A

2H7scFv-Ig cDNA and predicted amino acid sequence:

```

HindIII      NcoI      2H7 VL Leader Peptide→
-----
1  AAGCTTGCCG CC  ATGGATT TCAAGTCAG ATTTTCAGCT TCCTGCTAAT CAGTGCCTCA

                                2H7 VL→
61  V I I A R G Q I V L S Q S P A I L S A S
    GTCATAATTG CCAGAGGACA AATTGTTCTC TCCAGTCTC CAGCAATCCT GTCTGCATCT
    P G E K V T M T C R A S S S V S Y M H W
121 CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

                                BamHI
                                -----
181 Y Q Q K P G S S P K P W I Y A P S N L A
    TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATT ATGCCCATC CCACTGGCT
    S G V P A R F S G S G S G T S Y S L T I
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC
    S R V E A E D A A T Y Y C Q Q W S F N P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

                                (Gly4Ser)3 Linker
061 P T F G A G T K L E L K G G G G S G G G
    CCCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAGGTG GCGGTGGCTC GGGCGGTGGT

                                2H7 VH→
421 G S G G G G S S Q A Y L Q Q S G A E L V
    GGATCTGGAG GAGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGC TGAGCTGGTG
    R P G A S V K M S C K A S G Y T F T S Y
481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTCTG GCTACACATT TACCAGTTAC
    N M H W V K Q T P R Q G L E W I G A I Y
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCTGG AATGGATTGG AGCTATTATT
    P G N G D T S Y N Q K F K G K A T L T V
601 CCAGGAATG GTGATACTTC CTACATCAG AAGTCAAGG GCAAGGCCAC ACTGACTGTA
    D K S S S T A Y M Q L S S L T S E D S A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGGG
    V Y F C A R V V Y Y S N S Y W Y F D V W
721 GTCTATTTC TGCAGAGAGT GGTGTACTAT AGTAACCTTT ACTGGTACTT CGATGCTGGG
  
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# FIG.1B

BclI  
-----human IgG1 Fc domain →

781 G T G T T V T V S D Q E P K S C D K T H  
GGCACAGGGA CCACGGTCAC CGTCTCTGAT CAGGAGCCCA AATCTTGTGA CAAAACCTCAC

841 T C P P C P A P E L L G G P S V F L F P  
ACATGCCAC CGTGCCACG ACCTGAACTC CTGGGGGAGC CGTCAGTCTT CCTCTTCCCC

901 P K P K D T L M I S R T P E V T C V V V  
CCAAAACCCA AGGACACCTT CATGATCTCC CGGACCCCTG AGGTACATG CGTGGTGGTG

961 D V S H E D P E V K P N W Y V D G V E V  
GAGGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG

1021 H N A K T K P R E E Q Y N S T Y R V V S  
CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGCTCAGC

1081 V L T V L H Q D W L N G K E Y K C K V S  
GTCTCTCACC TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGT CAAGGTCTCC

1141 N K A L P A P I E K T I S K A K G Q P R  
AACAAGCCCC TCCCAGCCCC CATCGAGAAA ACAATCTCCA AAGCCAAAGG GCAGCCCCGA

1201 E P Q V Y T L P P S R D E L T K N Q V S  
GAACCAAGG TGTACACCTT GCGCCCATCC CGGGATGAGC TGACCAAGAA CCAGGTACGC

1261 L T C L V K G F Y P S D I A V E W E S N  
CTGACCTGCC TGGTCAAGG CTCTATATCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT

1321 G Q P E N N Y K T T P P V L D S D G S F  
GGGAGCGCG AGAACAACTA CAGACCAAGC CCTCCCGTGC TGGACTCCGA CGGCTCTCTC

1381 F L Y S K L T V D K S R W Q Q G N V F S  
TTCTCTTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA

1441 C S V M H E A L H N H Y T Q K S L S L S  
TGTCCTGTGA TGCATGAGGC TCTGCACAC CACTACAGCG AGAAGAGCCT CTCCTCTGCT

XbaI  
-----

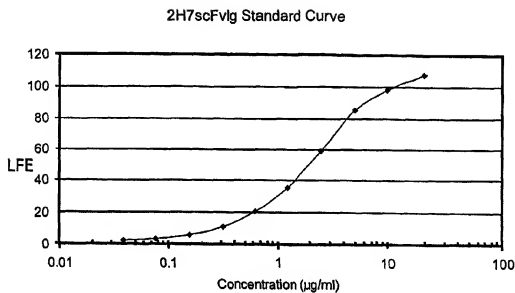
1501 P G K \* S R  
CCGGTAAAT GATCTAGA

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**FIG.2**



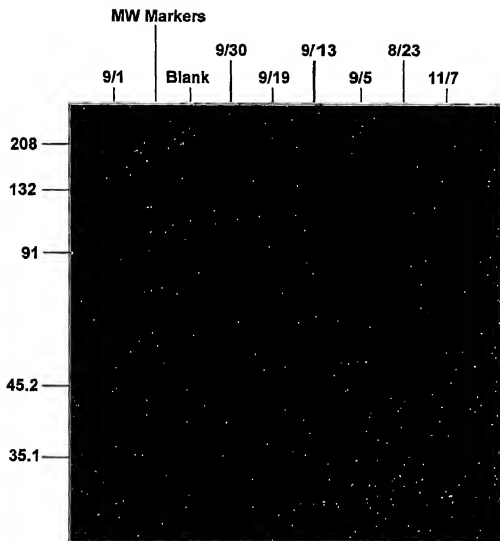
Clone	LFE @ 1:50	Estimated Concentration (µg/ml)
D2	26.1	56
IIIIC6	25.7	55
IVA3	28.6	61
Spent bulk	29.6	64

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**FIG.3**



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## FIG. 4A

**Complement Mediated B Cell Killing After Binding of CD20-targeted 2H7 Derivatives:**

2H7scFv-Ig Concentration	RAMOS	BJAB
20 µg/ml + complement	0.16	0.07
5 µg/ml + complement	0.2	N.D.
1.25 µg/ml + complement	0.32	0.1
Complement alone	0.98	0.94

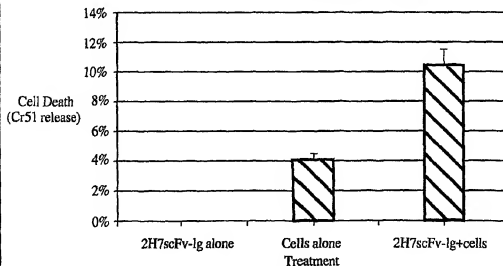
\*Viability was determined by trypan blue exclusion and is tabulated as the fraction of viable cells out of the total number of cells counted.

\*\*N.D. (not determined).

## FIG. 4B

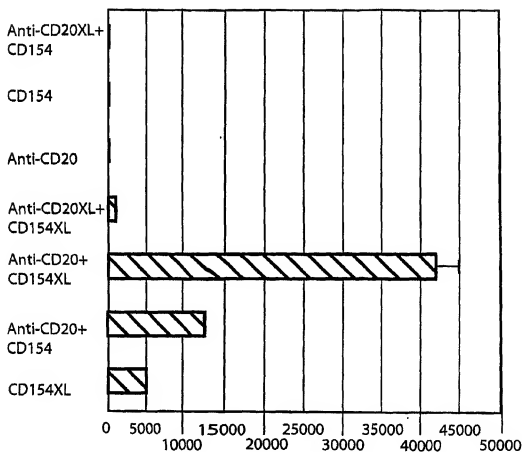
**Antibody-dependent cellular cytotoxicity (ADCC) mediated by 2H7scFv-Ig:**

**ADCC Activity of 2H7scFv-Ig on Ramos Cells**



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**FIG. 5**

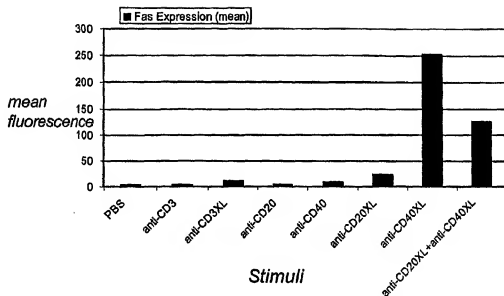


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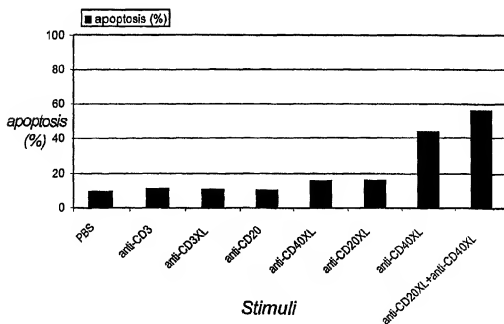
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**FIG.6A**



**FIG.6B**



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# FIG. 7A

2H7-CD154 L2 cDNA and predicted amino acid sequence:

```

HindIII      NcoI  2H7 VL Leader Peptide →
-----      -
1  AAGCTTGGCCG CC  ATGGATTTC TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

                2H7 VL →
      V I I A R G Q I V L S Q S P A I L S A S
61  GTCATAATTC CCAGAGGACA AATGTGTTCTC TCCAGTCTC CAGCAATCCT GTCTGCATCT

      P G E K V T M T C R A S S S V S Y M H W
121 CCAGGGGAGA AGGTCACAAT GACTGCGAGG GCCAGCTCAA GTGTAAGTTA CATGCACCTGG

                BamHI
                -----
      Y Q Q K P G S S P K P W I Y A P S N L A
181 TACCAAGCAGA AGCCAGGATC CTCGCCCAAA CCCTGGATTTC ATGCCCCATC CAACCTGGCT

      S G V P A R F S G S G S G T S Y S L T I
241 TCTGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTACTC TCTACAATC

      S R V E A E D A A T Y Y C Q Q W S F N P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

                                (Gly4Ser)3 Linker →
      P T F G A G T K L E L K G G G G S G G G
361 CCCACGTTGG GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

                2H7 VH →
      G S G G G G S S Q A Y L Q Q S G A E L V
421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

      R P G A S V K M S C K A S G Y T F T S Y
481 AGGCTTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

      N M H W V K Q T P R Q G L E W I G A I Y
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGCGCTGG AATGGATTGG AGCTATTATAT

      P G N G D T S Y N Q K F K G K A T L T V
601 CCAGGAATGG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

      D K S S S T A Y M Q L S S L T S E D S A
661 GACAAATCCT CCAGCACAGC CTACATGCGAG CTCAGCAGCC TGACATCTGA AGACTCTGGG

      V Y F C A R V V Y Y S N S Y W Y F D V W
721 GTCTATTCTT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGTACTCT CGATGTCTGG
  
```

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# FIG.7B

human CD154/amino acid 48→

Bcl/Bam hybrid site

781 | G T G T T V T V S D P R R L D K I E D E  
GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAAGAGGT TGGACAAGAT AGRAGATGAA

841 R N L H B D F V P M K T I Q R C N T G E  
AGGAATCTTC ATGAAGATTT TGTATTCTAG AAAACGATAC AGAGATGCAA CACAGGAGAA

901 R S L S L L N C E E I K S Q F E G F V K  
AGATCCTTAT CCTTACTGAA CTGTGAGGAG ATTAAAAGCC AGTTTGAAGG CTTTGTGAAG

BclI

961 D I M L N K E E T K K E N S F E M Q K G  
GATATAATGT TAAACAAAGA GGAGACGAAG AAAGAAAACA GCTTTGAAAT GCAAAAAGGT

BclI

1021 D Q N P Q I A A H V I S E A S S K T T S  
GATCAGATTC CTCAAATGTC GGACATGTC ATAAGTGAGG CCAGCAGTAA AACACATCT

1081 V L Q W A E K G Y Y T M S N N L V T L E  
GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT ACCCTGGA

1141 N G K Q L T V K R Q G L Y Y I Y A Q V T  
AATGGGAAAC AGCTGACCGT TAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII

1201 F C S N R E A S S Q A P F I A S L C L K  
TTCTGTTCCA ATCGGGAAGC TTGAGTCAA GTCCATTTA TAGCCAGCCT CTGCTAAAG

1261 S P G R F E R I L L R A A N T H S S A K  
TCCCCCGSTA GATTGAGAG AATCTTACT AGAGCTGCAA ATACCCACAG TTCCGCCAA

1321 P C G Q Q S I H L G G V F E L Q P G A S  
CCTTGCGGGC AACAATCCAT TCATTGGGA GGAGTATTG AATTGCAACC AGGTGCTTG

NcoI

1381 V F V N V T D P S Q V S H G T G F T S F  
GTGTTTGCA ATGTACTGA TCCAAGCCAA GTGAGCCATG GCATGGCTT CACGTCTTT

XhoI XbaI

1441 G L L K L E \* \* S R  
GGCTTACTCA AACTCGAGTG ATAATCTAGA



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# FIG. 7C

2H7scFv-CD154 S4 cDNA and predicted amino acid sequence:

```

HindIII      NcoI
-----      -
2H7 VL Leader Peptide →
      M D P Q V Q I F S F L L I S A S
1 AAGCTTGCCG CC ATGGATT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCCTCA

      2H7 VL →
      V I I A R G Q I V L S Q S P A I L S A S
61 GTCATAATTG CCAGAGGACA AATGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

      P G E K V T M T C R A S S S V S Y M H W
121 CCAGGGGAGA AGGTACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

      BamHI
-----
      Y Q Q K P G S S P K P W I Y A P S N L A
181 TACCAGCAGA AGCCAGGATC CTCCCCCAA CCTGGATT TATGCCCATC CAACCTGGGT

      S G V P A R F S G S G S G T S Y S L T I
241 TCTGGAGTCC CTGCTGCTT CAGTGGCAGT GGTCTGGGA CCTCTTACTC TCTCAACATC

      S R V E A E D A A T Y Y C Q Q W S F N P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTITAACCCA

      (Gly4Ser)3 Linker →
      P T F G A G T K L E L K G G G G S G G G
361 CCCACGTTG GTGCTGGAC CAAGCTGGAG TGAAAGGTG GCGGTGGCTC GGGCGGTGGT

      2H7 VH →
      G S G G G G S S Q A Y L Q Q S G A E L V
421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

      R P G A S V K M S C K A S G Y T F T S Y
481 AGGCCTGGG CCTCAGTGA GATGCTCTG AAGGCTTCTG GCTACACATT TACCAGTTAC

      N M H W V K Q T P R Q G L E W I G A I Y
541 AATATGCACT GGTAAAGCA GACACCTAGA CAGGCTCTGG AATGATTTGG AGCTATTATT

      P G N G D T S Y N Q K F K G K A T L T V
601 CCAGGAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

      D K S S S T A Y M Q L S S L T S E D S A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGGG

      V Y F C A R V V Y Y S N S Y W Y F D V W
721 GTCTATTICT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGGTACTT CGATGCTGCG
  
```

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# FIG.7D

human CD154/amino acid 108 →

```

                                Bcl/Bam hybrid site          BclI
781  G T G T T V T V S D P E N S F E M Q K G
    GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAGAAAACA GCTTTGAAAT GCAAAAAGGT

    BclI
    ~~~~~
841  D Q N P Q I A A H V I S E A S S K T T S
    GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACACATCT

    V L Q W A E K G Y Y T M S N N L V T L R
901  GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTGGT AACCTGGAA

    N G K Q L T V K R Q G L Y Y I Y A Q V T
961  AATGGGAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

                                HindIII
                                ~~~~~
1021 F C S N R E A S S Q A P F I A S L C L K
    TTCTGTTCCA ATCGGGAAGC TTCGAGTCRA GCTCCATTTA TAGCCAGCCT CTGCTTAAG

    S P G R F E R I L L R A A N T H S S A K
1081 TCCCCCGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCGCCAAA

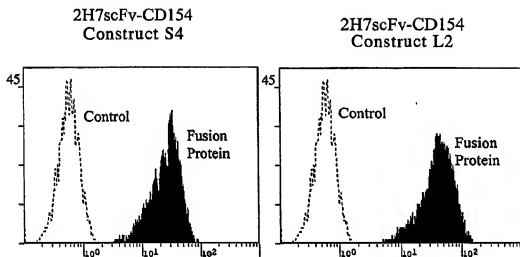
    P C G Q Q S I H L G G V F E L Q P G A S
1141 CCTTGGGGC AACATCCAT TCACCTGGGA GGAGTATTG AATTGCAACC AGGTGCTTCG

                                NcoI
                                ~~~~~
1201 V F V N V T D P S Q V S H G T G F T S F
    GTGTTTGTA ATGTGACTGA TCAGAGCCAA GTGAGCCATG GCACTGGCCT CACGTCCTTT

                                XhoI          XbaI
                                ~~~~~
1261 G L L K L E * * S R
    GGCTTACTCA AACTCGAGTG ATAATCTAGA
  
```

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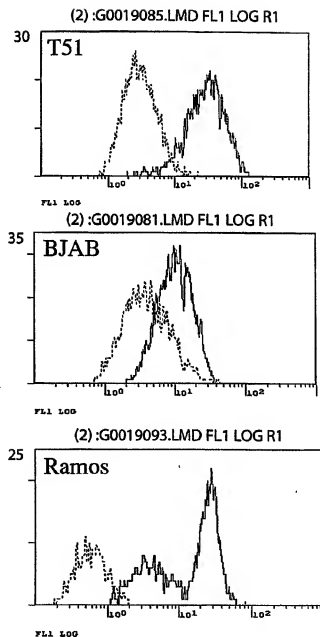
**FIG.8**



CD20 CHO cell targets + (control or fusion protein)  
+ Biotin-CD40Ig + PE-SA

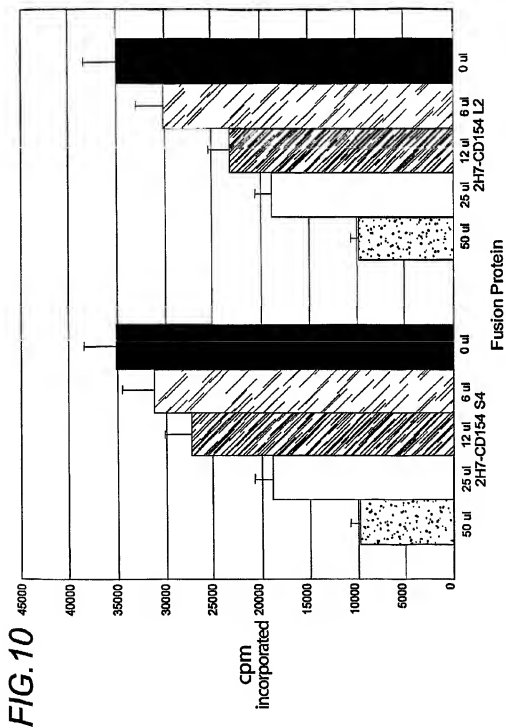
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**FIG.9**



.....control supernatant    2H7scFv-CD154 supernatant

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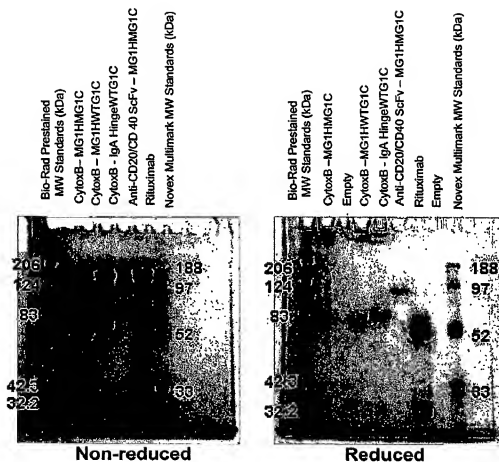


**FIG. 11**



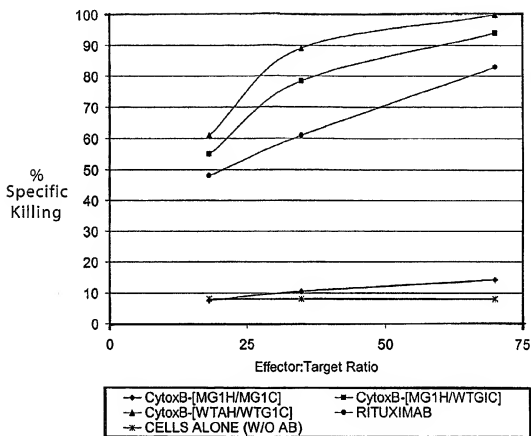
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**FIG. 12**



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FIG. 13



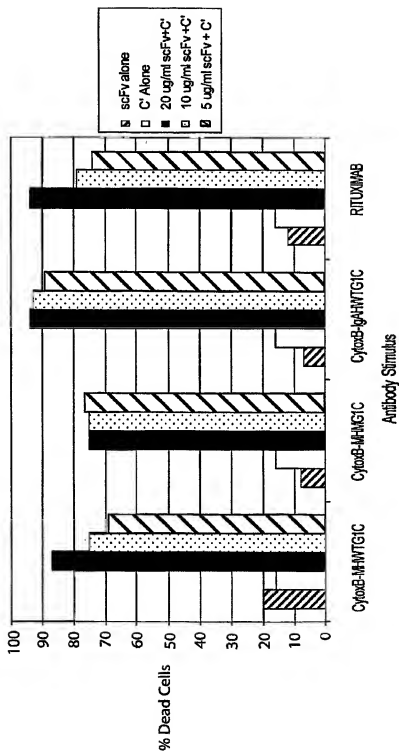


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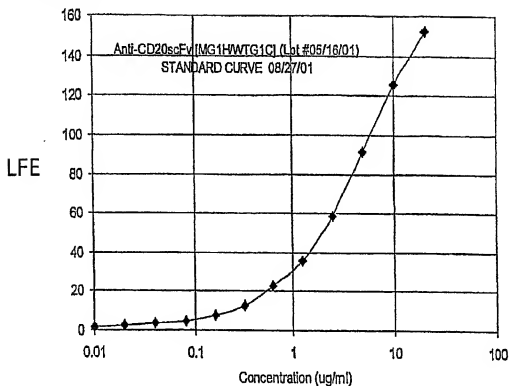
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FIG. 14



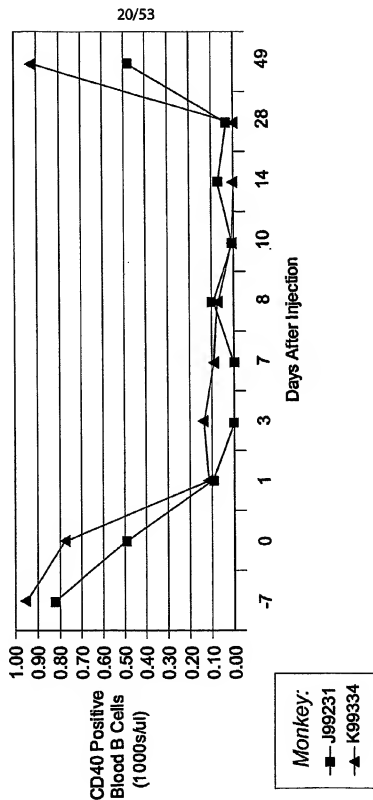
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FIG. 15



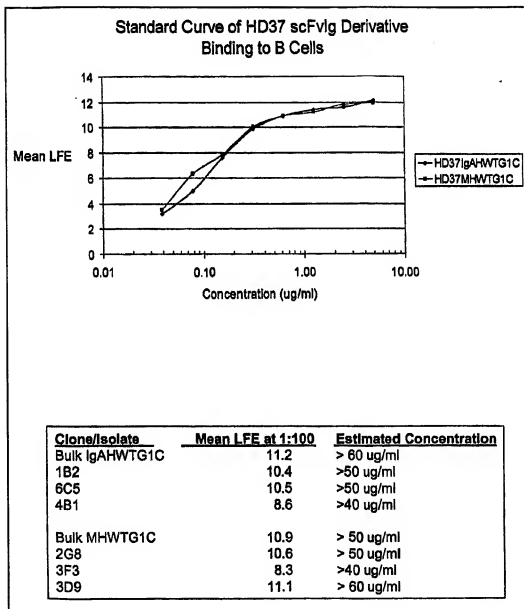
	Day	Monkey J99231		Monkey K99334	
		LFE(1:40)	Concentration ( $\mu$ g/mL)	LFE(1:40)	Concentration ( $\mu$ g/mL)
Injection $\rightarrow$	-7	2.41	<0.6 $\mu$ g/mL	1.51	<0.4 $\mu$ g/mL
	0	2.22	<0.6 $\mu$ g/mL	1.63	<0.4 $\mu$ g/mL
	1	73.8	220 $\mu$ g/mL	44.4	100 $\mu$ g/mL
Injection $\rightarrow$	3	20.0	28 $\mu$ g/mL	40.2	80 $\mu$ g/mL
	7	15.6	24 $\mu$ g/mL	15.7	24 $\mu$ g/mL
	8	39.1	80 $\mu$ g/mL	42.6	92 $\mu$ g/mL
	10	11.5	18 $\mu$ g/mL	2.74	1.2 $\mu$ g/mL
	14	2.05	0.6mg/mL	1.96	0.6 $\mu$ g/mL

FIG. 16



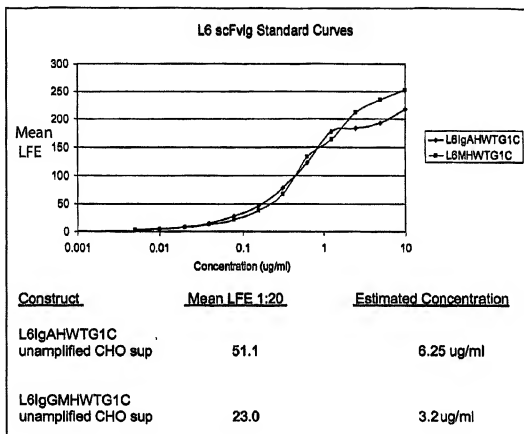
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**FIG. 17**



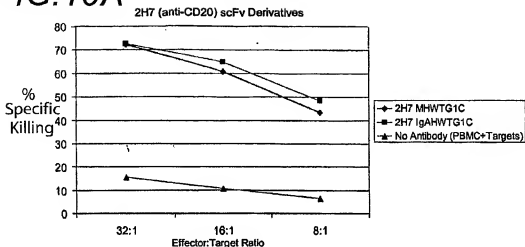
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**FIG.18**

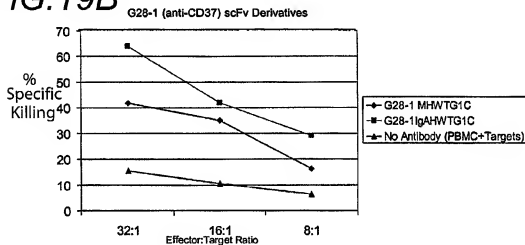


**FIG. 19A**

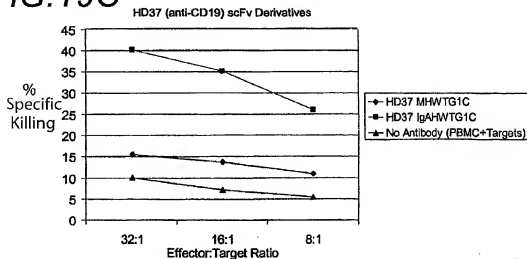
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**FIG. 19B**

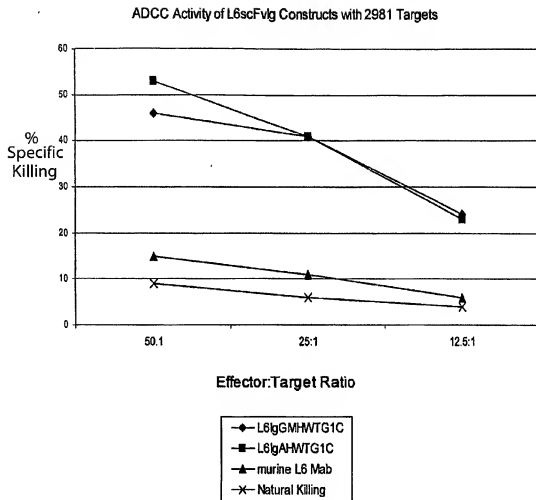


**FIG. 19C**



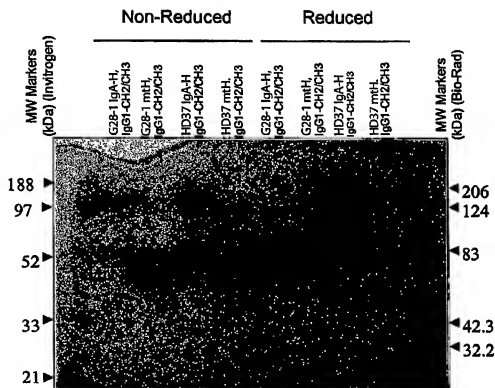
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**FIG.20**



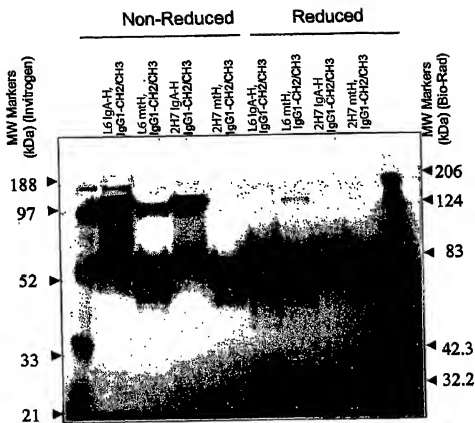
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**FIG.21**





**FIG. 22**



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FIG.23

Hinge

CH2

Human IgG1: -----HTCPPC P APELLSGPSVFLPPPKPDITMISRTPEVTCVVVDVSHEDPEVKFNWYDG  
Llama IgG2: DQEPKTPRPQPPQPNPTTESKCFKC P APELLSGPSVFLPPPKPDVLSISGRPEVTCVVVDVGDPEVSNWYIDG  
Llama IgG1: --EPHGG-----CTCPQC P APELLSGPSVFLPPPKPDVLSISGRPEVTCVVVDVGDPEVSNWYIDG  
Llama IgG3: --AHHSEDT-----SKCFKC P APELLSGPTVFIFPPPKADWLISITRKPEVTCIMWTWVKKTIIRSSSSNSVDD

CH3

VEVHNAKTFRREEQNSTYRVVSVLPIQHQDWLNGKEYCKCKVNKKALPAPIERTISAKAGQTRPEQVYITLAPSRDELTRKQVSLT  
TAEVTRANTRPKEEQFNSTYRVVSVLPIQHQDWLNGKEYCKCKVNKKALPAPIERTISAKAGQTRPEQVYITLAPHREELAKDTVSVT  
VEVTRANTRPKEEQFNSTYRVVSVLPIQHQDWLNGKEYCKCKVNKKALPAPIERTISAKAGQTRPEQVYITLAPHREELAKDTVSVT  
TEVHTAETKRPKEEQFNSTYRVVSVLPIQHQDWLNGKEYCKCKVNKKALPAPIERTISAKAGQTRPEQVYITLAPHREELAKDTVSVT

CLVKGFTPSDIAVEWESNGQPEN--NYKTTTPPVLDSDGSFFLYSKLTVDKSNWQQGNVFSQSWMEALHNHYTQKSLSLSPGK  
CLVKGFTPPDINVEWQRNGQPSSEGTANTPTQLINDGTFFLXSKXSVGKNTWQQGSETTCVVMHEALHNHYTQKSITVSSGK  
CLVKGFTPADINVEWQRNGQPSSEGTANTPTQLINDGTFFLYSKLSVGKNTWQRGETLTGVVMHEALHNHYTQKSITVSSGK  
CLVKGFTPADINVEWQRNGQPSSEGTANTPTQLINDGTFFLYSKLSVGKNTWQQGSEVFTCVVMHEALHNHSTQKSITVSSGK

**FIG.24**

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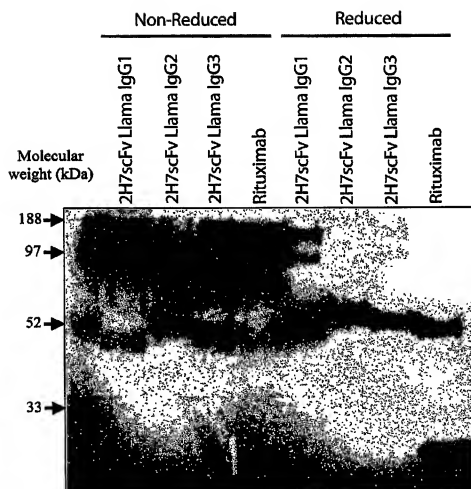
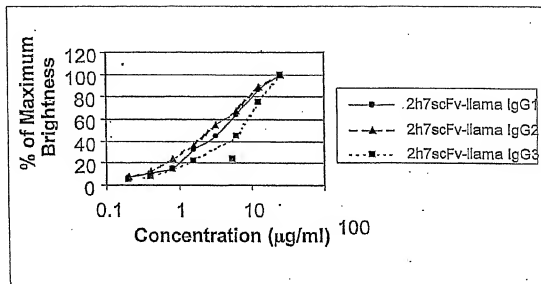
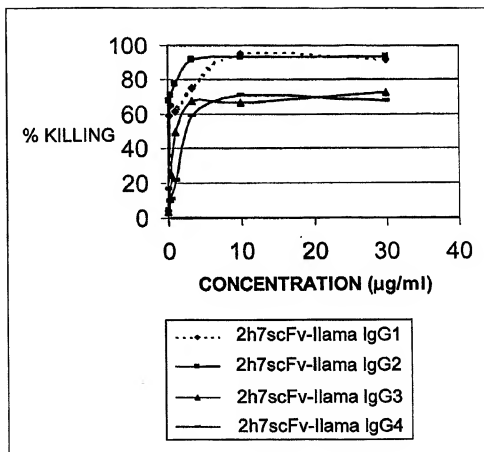


Figure 25



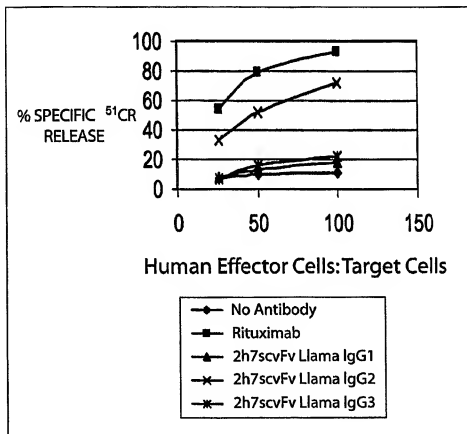
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**FIG.26**



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**FIG.27**



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**FIG.28**

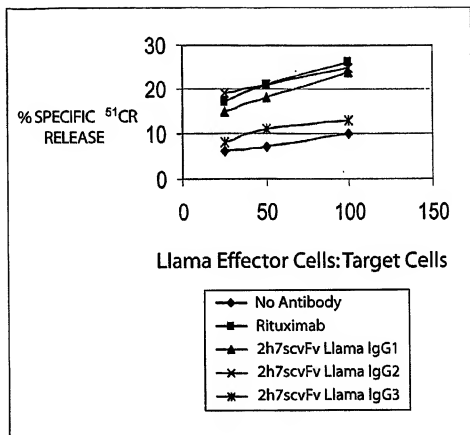
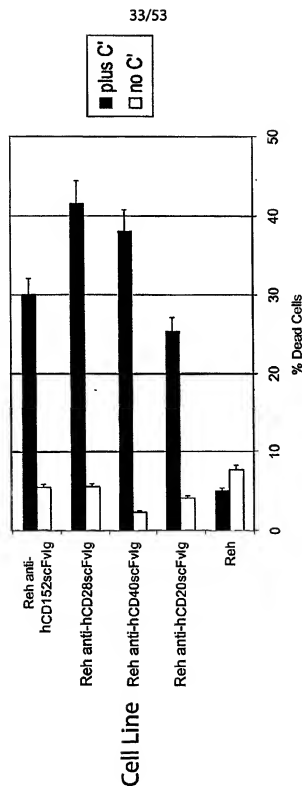


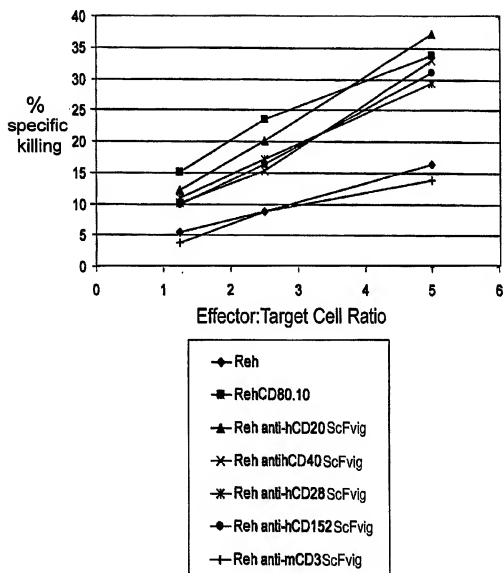
FIG. 29





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FIG. 30

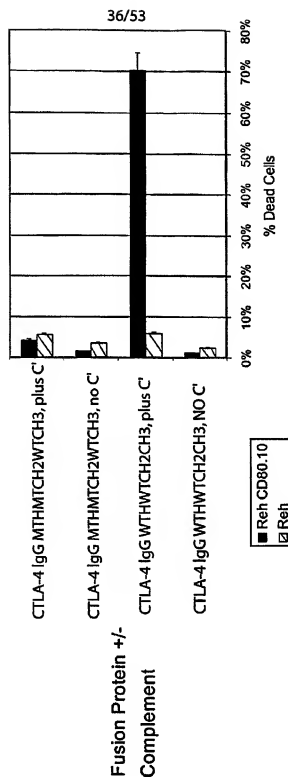


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FIG.31

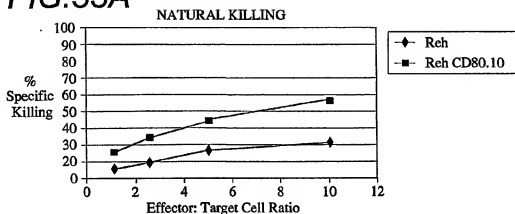
Name Identifier	Hinge Sequence	CH2 Sequence	CH3 Sequence	SEQ ID NO:
IgG WTH (CCC) WTCH2CH3	IgG1 WT Hinge (CCC)	Wild Type CH2	Wild Type CH3	
IgG MTH (SSS) WTCH2CH3	IgG1 Mutant Hinge (SSS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
VH SER 11 IgG MTH (SSS) WTCH2CH3	IgG1 Mutant Hinge (SSS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
IgG (SSC) WTCH2CH3	IgG1 Mutant Hinge (SSC)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
IgG (SCS) WTCH2CH3	IgG1 Mutant Hinge (SCS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
IgG (CSS) WTCH2CH3	IgG1 Mutant Hinge (CSS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
IgG MTH(SSS) MTCH2WTCH3	IgG1 Mutant Hinge (SSS)	Mutant CH2 (IgG1) Pro ? Ser 238	Wild type CH3 (IgG1)	
IgAH IgGWTCH2CH3	IgA Hinge	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
IgAH IgACH2CH3	IgA Hinge	Wild type CH2 (IgA)	Wild type CH3 (IgA)	
IgAH IgA-T 4	IgA Hinge	Wild type CH2 (IgA)	Truncated CH3 (IgA) (deletion of 4 amino acids at carboxy terminus)	

FIG.32

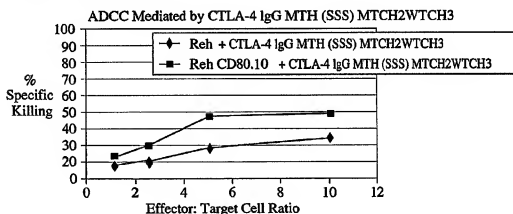


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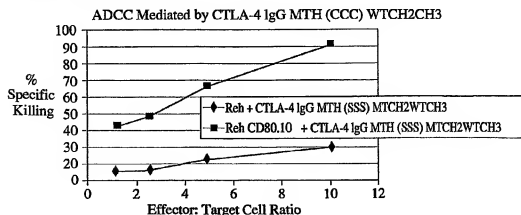
**FIG.33A**



**FIG.33B**

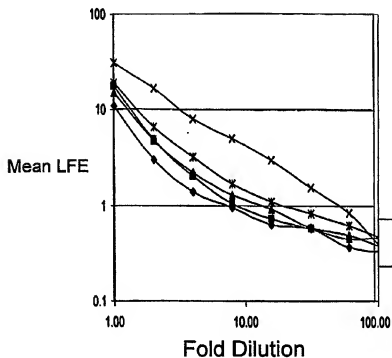


**FIG.33C**



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**FIG.34**



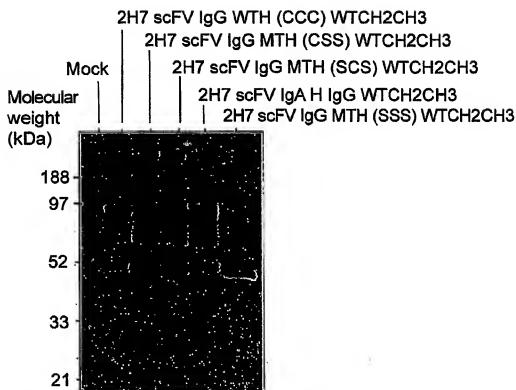
- ◆ 2H7 scFv WTH (CCC) WTCH2CH3
- 2H7 scFv MTH (CSS) WTCH2CH3
- ▲ 2H7 scFv MTH (SCS) WTCH2CH3
- \* 2H7 scFv MTH (SSC) WTCH2CH3
- ✱ 2H7 scFv VH11SER WTH (CCC) WTCH2CH3

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# FIG.35

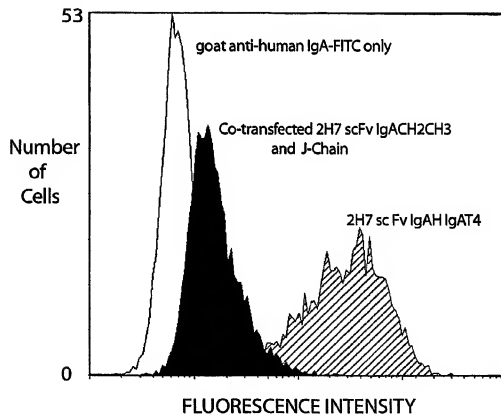


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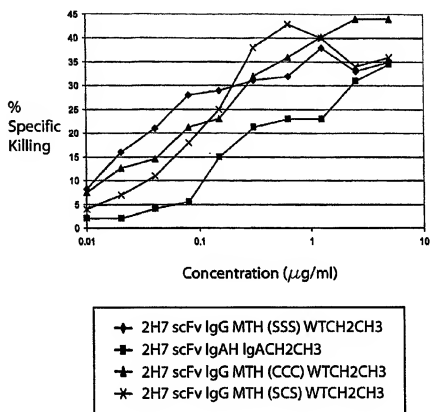
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**FIG.36**



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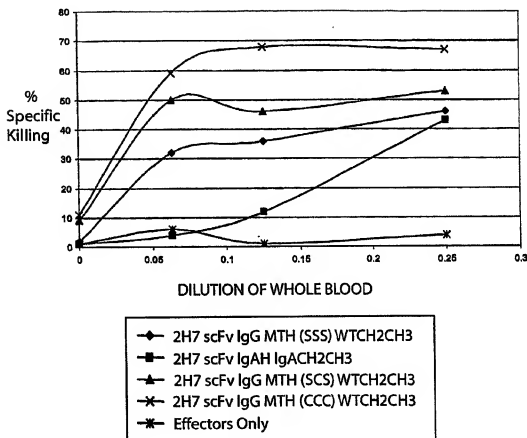
**FIG.37**





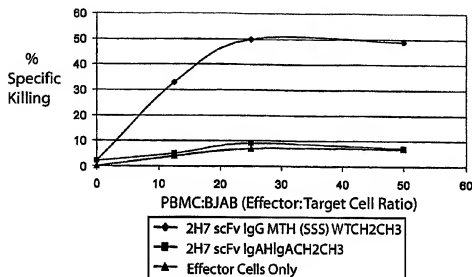
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FIG. 38

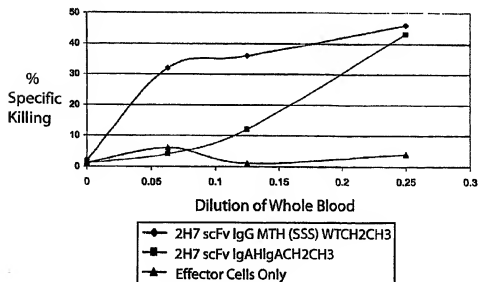


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**FIG.39A**



**FIG.39B**



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**FIG. 40**

